MASS CULTURE OF ALGAE

A Thesis Submitted
In Partial Fulfilment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

By

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to the

DEPARTMENT OF CHEMICAL ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY KANPUR

DECEMBER, 1976

CHE-1976-D-MIS-MAS

CERTIFICATE

Certified that the work "Mass Culture of Algae" has been carried out under my supervision and that it has not been submitted elsewhere for a degree.

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ACKNOWLEDGEMENT

The author expresses his deep sense of gratitude to Professor C.V. Seshadri whose guidance and encouragement has given shape to this work.

Thanks are due to Dr. B.L. Amla, Director, Central Food Technological Research Institute, Mysore, Dr. D.N. Saraf, Head, Department of Chemical Engineering, Departmental Post Graduate Committee, Department of Chemical Engineering and Senate Post Graduate Committee, I.I.T., Kanpur, for allowing me to do the experimental part of my research work at C.F.T.R.I., Mysore.

This thesis was completed because Dr. W. Becker, Leader, Indo-German Algal Project offered complete facilities, help and encouragement to the author to work at Mysore. Sincere and grateful thanks are due to him. Thanks are also due to Shri P.K. Ramanathan, Project Coordinator, Process Development, for his keen interest and continuous help.

The author's special thanks are to Dr. G.S. Venkataraman for his helpful discussion and training, which the author was given at Department of Microbiology, Indian Agricultural Research Institute, New Delhi.

The author acknowledges the help and cooperation rendered by the staff of Chemical Engineering Department, I.I.T., Kanpur, and Indo-German Algal Project, C.F.T.R.I., Mysore.

Author

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Algal species Scenedesmus acutus was cultivated in open elongated recirculation tanks agitated by paddle wheels in the laboratories of the Central Food Technological Research Institute, Mysore. Indoor illuminated tube cultures were also studied for the effect of various parameters on growth.

The parameters studied included among others:

1. Nutrient concentration and nitrogen/phosphorus ratio in indoor and outdoor cultures.

2. Hydrogen ion concentration in outdoor cultures and utilisation of bicarbonate by Scenedesmus acutus.

3. The effect of mixing and aeration on outdoor cultures.

4. The reuse of centrifuged clear medium in outdoor cultures.

5. The effect of inoculum size on growth in outdoor cultures and the determination of optimum inoculum size.

6. The effect of addition of organic carbon in the form of

molasses on outdoor and indoor cultures.

7. The estimation of dispersion coefficients from dye studies, and the rates of carbon dioxide desorption from clear liquid in outdoor cultures.

8. The effect of carbon dioxide feed methods in outdoor cultures.

9. Effect of harvesting time on protein content of the harvested algal mass.

The emphasis was on engineering studies and several interesting results for the proper design of large scale algal cultures have been obtained. These results are summarized first as follows:

1. In cultures that are not supplied an external source of pure carbon dioxide, the mixing of the culture alone is sufficient to supply oxygen at night, and to keep the algal cells in suspension.

2. The recycle of centrifuged clear medium (after harvesting) is not harmful for further growth in outdoor cultures.

3. There is an optimum initial concentration of 200 mg/l which gives the best yield over and above the initial biomass. This optimum is not a cost-effective optimum. It does not account for costs involved in inoculum cultivation, or harvesting and other costs.

4. Molasses in low concentrations can be used as a cheap organic carbon source for growth in the day time. When added

with carbon dioxide the effect of molasses on growth is cumulative to the effect of carbon dioxide.

5. The dispersion coefficient based on a two dimensional model (after G.I. Taylor), is related to the Reynolds number. The desorption coefficient for carbon dioxide (or decarbonation coefficient) in clear liquid (outdoors) is related linearly to the dispersion coefficient. The Sherwood number follows a Colburn type relationship with the Reynolds number based on hydraulic radius.

6. Pure carbon dioxide fed intermittently rather than continuously gives better growth rates and better efficiencies of carbon utilisation. This also results in considerable savings in gas usage. It appears that intermittently fed carbon dioxide is needed only for about 4/5 hours a day during the solar radiation peak period.

7. The harvesting time is a very important parameter for optimum protein recovery. Early morning harvesting gives 10-15% more protein in the algal biomass.

The other results of importance are as follows:

1. The nitrogen and phosphorus concentration are not sensitive parameters in outdoor cultures. At low nitrogen concentrations the biomass yield is not different but the protein readjusts itself to the lowered nitrogen concentration.

2. Scenedesmus acutus utilise bicarbonate carbon as a carbon source outdoors. The cultures are not very sensitive to pH in outdoor conditions between tested range pH 7.5 and 9.5

without external supply of carbon dioxide, and between pH 6.5 and 7.5 with external supply of carbon dioxide.

These studies have shown that this algal species can be profitably cultivated in India for food using cheap sources of carbon, and in fairly easy-to-make tanks. A number of design criteria have emerged as a result of these studies:

1. Optimum depth for the growth of algae.
2. Optimum starting concentration of algae.
3. Efficiency of aeration and mixing system.
4. Use of organic carbon in the form of molasses.
5. Mode of nutrients (carbon dioxide and molasses) feeding.
6. Optimum harvesting time.
7. Carbon dioxide quantities and rates.
8. Gas transfer rates from the culture.
9. Heat Transfer rate in drying algae.
10. Physical properties e.g., density and viscosity of thick algal slurries.

PART I

CHAPTER 1

INTRODUCTION

The present status of development programmes on mass culture of algae is in a very critical stage. The opinions about the usefulness of such programmes oscillate between two extremes. One is shown by the statement 'Algal Cultures will open the ways to the coming industries of controlled photosynthesis' (Oswald, 1962), and the other extreme is the closing down of many such programmes. Present day economic criteria (which cannot be applied for food) is applied to evaluate the feasibility, and doubts are raised when it is calculated that large scale production is not immediately economically possible. Such an attitude is rather shortsighted. Algae are primary producers, simple in structure, easily accessible for genetic manipulation, and thus can play an outstanding role as easily controllable, highly efficient, industrial photosynthetic producers in solving many problems of food, fuel and pollution.

The fundamental difference between algal cultivation and agriculture is that former is an industrial photosynthetic process of higher efficiency, which can be a continuous process having better control on many factors. Photosynthetic production in agriculture is essentially a batch process, and of lower efficiency.

The algae can be envisaged as a possible source of food,

polysaccharides and pigments, absorbers of carbon dioxide, producers of oxygen in space vehicles, remover of pollutants, and fixers of solar energy. Calvin's idea (Calvin 1976), of modifying higher plants (e.g., rubber trees which produce higher hydrocarbon) to produce high grade petroleum can be extended to envisage algae as a producer of high grade petroleum by genetic modification (may be crossbreeding of petroleum producing bacteria and algae). There are also algae that produce petroleum.

At present studies on mass culture of algae are focussed on the three following aspects:

1. Mass culture of algae for algal cells, and their components.
2. To control and/or to improve the environment.
3. To fix and store solar radiation.

This thesis is the report of an experimental study dealing with the first of these three aspects.

There is a widespread interest in growing algal cells for cell components. Algae contain 30-60% protein and can be used as food and fodder. The composition of the algae Scenedesmus acutus (used in this work), protein efficiency ratio, biological value, digestibility coefficient and net protein utilization are given in appendix 1. Appendix 2 shows the benefits of cultivation of algae for food.

Some of the algae can fix nitrogen of the atmosphere,

and can be used as fertilizer and soil conditioner. Algae are good source of nitrogen, potassium, and other organic compounds useful for higher plants. The increase in the yield of paddy due to algae inoculation is given in Appendix 3.

The lipids, carbohydrates, vitamins, amino acids, antibiotics, and pigments are some of the other products which can be recovered from algae. Most of the radioactive amino acids are produced by growing algae in radioactive nutrient media.

The second line of research concerns the use of algae in controlling and/or improving the environment. This includes waste water treatment, and bioregenerative systems. Algal cultures in treating waste waters are attractive due to their simplicity. In these systems algae are obtained as a by-product by slight modification of the process. Algal bioregenerative systems containing algae are used for carbon dioxide removal and oxygen regeneration in the enclosed environment of space vehicles.

Lastly algae containing photosynthetic pigments are capable of fixing solar radiations, and converting them into chemical energy. This chemical energy can be regenerated either by burning biomass directly or from gases which are produced by anaerobic digestion of algae.

These different systems have many specific and common features. The main feature of the process is to convert the solar energy into chemical energy of organic substances. The production of algae consists of two processes; The

photosynthesis in which the energy is accumulated, and the production process in which cell materials are produced by growth and cell division. The production of algae like other chemical processes is controlled by the concentration of reactants, their availability, and the environment. The reactants of importance for algal cultivation are radiation energy, nutrients and water. Products of the process are cell materials, extracellular compounds and oxygen. Extracellular products may be growth promotor or growth inhibitor .

Turbulence in algal cultures is important since this keeps the algae and nutrients in suspension, governs the absorption and desorption of gases, and the light-dark pattern of algae by mixing the algal cells at different depths and exposing them to light on the surface of the culture by cell movement.

The suspensions of algae from large scale cultures are very dilute containing less than 0.1% of algal biomass. A number of methods for harvesting algae such as centrifugation, precipitation, filtration, and sedimentation have been tested by many workers. Economical harvesting of the algal cultures is still ' unsolved problem. Thus there are two main problems in cultivating algae on mass scale; 1. To provide optimum environment for the growth of right type of algae, and 2. To harvest the culture.

This thesis on mass cultivation of algae is divided into two parts: Part I consists of a literature review and

experiments on physico-chemical properties, followed by an analysis of the gross parameters of the system. In this section the direct results are reported. In some cases these results came from measurements in an experiment specially carried out for that purpose. In others they came out of experiments carried out for other end results. In all cases the results are direct measurements.

The second part of the thesis describes the experiments involving the fine parameters of the algal systems. This includes the effect of fixed carbon addition at night on the growth of algae, mixing parameters, carbon dioxide transfer, effect of carbon dioxide feed methods, and the effect of harvesting time on protein content. The use of words 'gross' and 'fine' in describing the parameters is arbitrary, and is merely for convenience. This thesis has been carried out for studying the engineering of algal systems and all the parameters are equally important.

A brief literature review opens the thesis. This includes the uses of algae, different algal systems, and the work done on algae in India. A short general bibiliography is given at the end of Chapter 2, and the major references are given at the end of the chapter dealing with that topic. In Chapter 3, general methods and materials used are described. Specific methods used in the experiments are described in the chapter dealing with a particular experiment. Each chapter starts with a brief introduction, discusses the previous work, experimental methods, results, discussion and conclusions.

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1. Calvin, M. 1976. American Scientist, 64, 3: 270.

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CHAPTER 2

LITERATURE REVIEW

Algae can be defined as a heterogeneous collection of cryptogamic plants, belonging to sub-group Thallophyta, most of which are aquatic in nature, possess chlorophyll and are less specialized in structure than higher plants. Algae can grow in a wide variety of environments, can fix solar radiation and nitrogen of the air, can synthesize protein, and convert inorganic carbon to organic carbon through photosynthesis. Algae are considered as a possible solution for the worsening problem of food, fuel and fertilizer supply. This triple-fold use of algae as food, fuel and fertilizer has brought it again in focuss. Higher growth rate and photosynthetic efficiency, easy to maintain optimum conditions, easy accessability for genetic modification, are some of the advantages of using algae in industrial photosynthesis. Research and development have been going on for quite some time, and there is an extensive literature.

2.1. LITERATURE

The literature on physiology, biochemistry and cultivation of algae have been reviewed by Krauss (1958, 1962) and Tamiya (1957), and several volumes (Burlew (1953), Kachroo (1960), Lewin (1962), Jackson (1968), Venkataraman (1969) and Stewart (1974)) have been published. Burlew's 'Algal Culture from Laboratory to Pilot Plant' is a brilliant treatment of the basic problem of mass culture. The work done at Trebon, Czechoslovak

Academy of Sciences is reported in the form of Annual reports of the Laboratory of Algology. The literature collected and presented on cards by Phycological Documentation Center, Kohlenstoffbiologische Forschungsstation, Dortmund, Federal Republic of Germany, is a very good source of information on algae. The Phycological Documentation Center uses a numerical documentation code of two levels, which can be processed by computer retrieval method (see Javornicky et al. 1973 for details). The work done on algae in the field of pollution and water resources is published by IFI/Plenum Data Corporation in cooperation with Water Resources Scientific Information Service, USA, in the form of two volumes named 'Algae Abstracts'. Volume 1 compiles the work upto 1969, and Volume II from 1969 to 1972. Each volume including computerized author index is a self contained entity, offering ready access to the literature for the time given. From the references given it seems that two abstracting agencies do not have any interaction.

2.2. USES OF ALGAE

2.2.1. ALGAE AS PROTEIN SOURCE - Algae have been used as a food for a long time. The natives of Chad in Africa and Aztecs in Mexico have been using it as a part of their diet. In Japan 20 different kinds of algae are used in food, and algal extracts can be used in ice creams, condiments, etc. Clinical trials have been carried out in Germany, Mexico and Thailand, and it has been found fully acceptable.

The particular appeal of algae as a food source is that

these are primary producers. Unicellular algae are smallest and simplest photosynthetic apparatus, and thus are expected to have higher metabolic rates and nucleic acid contents. Vincent (1971) has discussed the advantages and disadvantages of growing algae for food. Other reviews on the subject are by Fisher (1953), Golueke et al.,(1959 a), Mathern (1965), Clement (1968), Lipinski (1974) and Vaslin (1975). Benefits of growing algae for food, and biological values, net protein utilization ratio and protein efficiency ratio of Scenedesmus accutus are given in Appendix 1 and 2.

High nucleic acid content, deep color which can mask every other colour, and high cost of production are some of the objections raised against cultivation of algae for food. Higher growth rates are associated with higher nucleic acid content, and compromise has to be made between the two. The growth rates so also nucleic acid contents of algae are between bacteria and yeast, and higher plants. Thus algae seems to be the best choice. The use of algae does not raise the uric acid levels as high as in the case of yeast and bacterial single cell protein. Algae contain 4 to 6% nucleic acid, and PAG (Protein Advisory Group, FAO, Rome, U.N.) limits the consumption of nucleic acid at 2 g/day per capita. This means that 32 gm of algae can be consumed, which is equal to 16 g protein per day per capita, and is still higher than the average diet at present. Kraut et al.,(1966), Soeder and Pabst (1975) and Vaslin et al. (1970) have reported the results of clinical and preclinical tests of algae. The colour and flavour may not be the serious

problems. Everybody eats green vegetables. There is no report in the literature that algae were unacceptable to common men who need protein. Preliminary tests at IGAP, CFTRI, Mysore have showed that there is no difference in taste and flavour in products containing 2% algae. At least nobody rejected the products for green colour. Algae have been used in Auroville (India) by some people for 1½ years, and group of people (under controlled conditions) for 4-6 weeks at a time.

Sewage grown algae can be used as animal feed. Experiments with rats and chicks have been reported by ~~Oswald and~~ Golueke & Oswald (1968 a), Grau and Klein (1957) and Leveille et al., (1962). Hintz et al., (1966, 1967) have reported that a mixture of barley, 10% algae and small quantity of vitamin B_{12} fed to swine resulted in weight gain equal to those attained with control on barley and fish meal.

2.2.2. ALGAE IN SEWAGE TREATMENT - Environmental engineers have been working on algae because it affects them in many ways. Algae change the colour of water, spoil the recreational areas clog the water treatment filters, super saturate and deplet the dissolved oxygen of rivers and lakes in day and night resulting in abundance and death of fish. On the other hand they are water purifier, and treatment in oxidation ponds is mainly achieved through photosynthesis by algae. Algae and bacteria work together symbiotically. In waste treatment systems algae is a by-product and can be obtained in most cases by slight modification of the process.

Environmental protection agency, the United States Bureau of Reclamation and California Department of Water Resources (1971) combined together toinvestigate the possibilities of removing nitrate by algal systems. California department of water resources and US environmental protection agency have done extensive work on eutrophication. 'Algae Abstracts' published by IFI/Plenum Data Corporation is an excellent source of literature on the work done.

2.2.3. ALGAE AS FERTILIZER - Algal species can contribute in several ways towards the improvement of crop yield 1) by fixation of atmospheric nitrogen and supply of extracellular nitrogen compounds towards the plant nutrition, 2) by supply of essential plant chemicals (hormones, etc.) and 3) by improvement of soil quality. Seshadri (1970) has discussed the economical and social benefits of using algae as fertilizer. In Japan, USSR, and other Asian countries the algae have been used as green manure. The increase in yield due to algal inoculation has been shown in Japan, China and India. Indian Agricultural Research Institute, New Delhi, Central Rice Research Institute, Cuttack, Banaras Hindu University, Varanasi, and Bhabha Atomic Research Centre, Bombay have been working in India on the utilization of algae as biofertilizer. The other literature references are Willis and Green (1948), De and Mandal (1956), Singh, R.N. (1961), Venkataraman (1962), and Sankaram (1971). R.N. Singh's monograph 'Role of Algae in Nitrogen Economy of Indian Agriculture' is an excellent treatment of the subject. G.S. Venkataraman's group (IARI) has done an

extensive work on algal inoculation of paddy fields; Venkataraman (1967, 1972). Subramanyam (1971) has reviewed the work done on blue green algae in relation to agriculture. Appendix 3 shows the benefits of algae inoculation.

2.2.4. ALGAE AS A SOURCE OF ENERGY - Energy crisis in recent years has changed the economy of almost all the countries. The research and development programmes on solar energy utilization have received utmost attention, and are on the top priority of national research program of many countries. High capital cost, need for highly developed technology and highly trained personnel, the high expense on plant and personnel safety, hazards of nuclear accidents, and the problem of maintenance of high standards of waste disposal make nuclear energy prohibitive for developing countries. The above limitations of nuclear power, and simplicity of algal systems have diverted the global attention to algal systems for fixation of solar energy.

The feasibility of the methane fermentation of algae has been proved in laboratories (Golueke et al., 1957; Golueke and Oswald, 1959 c; Saini, 1971). Oswald and Golueke (1960) have analysed and described the feasibility and advantages of hypothetical solar energy conversion systems using algae.

2.2.5. ALGAE IN BIOREGENERATIVE SYSTEMS - Probably most familiar and efficiently supported research programme on algae is bioregenerative system (Golueke et al. 1964, and Lisouskij 1964). The system size, weight, efficiency and reliability

are the main problems in designing a bioregenerative system. Hannan and Constance (1963) have analysed the reliability of photosynthetic gas exchangers. The other literature references on the subject are: Myers (1964 a, 1964 b); Bongers, et al., (1958), Dyer, et al. (1963); Shelef, et al., (1970); Fredrickson, (1961); Leone (1961); Matthern (1964) and Rabe and Benoit (1962). Miller and Ward (1965) have reviewed the work done upto 1964 on regenerative systems, and have tabulated the data on algal propagators up to 1964.

2.2.6. OTHER USES - Algae may be used as a source of raw materials for many other processes. This has been reviewed by Fisher et al., (1953). The possibility of growing algae for sterol production is analysed by Krauss and McAleer (1953). Many polysaccharides can be recovered from marine algae. Alginic acid, laminarin, fucoidin, galactans, agar, carrageenin, xylans and mannan are some of the important polysaccharides. Many pigments e.g., chlorophylls and phycobilins can be produced, but only carotenoids having pro-vitamin A activity are of current interest (Volskey et al., 1970). Possible uses of algae are discussed by Milnen and Fischer, Fisher and Burlew, and Krauss et al.,(1953).

2.3. NUTRITION OF ALGAE

The literature on the mineral nutrition of algae is reviewed by Burlew (1953), Krauss (1958), Provasoli (1958), Lewin (1962), Gerloff (1963), Eyster (1965, 1968),

Nicholoss (1963), Hunter and Provasoli (1964), Healey (1973) and O'Kelley (1968, 1974). The different nutrient formulations are published by Lancaster and Tischer (1962), Burlew (1953), and Venkatraman (1969). O'Kelley (1974) has reviewed the macro nutrients S, P, Ca, Mg and micro nutrient elements Fe, Mn, Cu, Zn, Mo, Cl, Co, B, Si, Na, Vn and I requirements of algae. Vitamins and growth regulators of algae are examined by Provasoli and Carlucci (1974). The effect of the form of nitrogen (NH_3 or NO_3) is studied by Tischer and Davis (1971). The role of carbon and phosphorous on the eutrophication is reviewed by Goldman (1972) and Brown (1972), respectively. A standard method to find out the effect of nutrient media has been outlined by Environmental Protection Agency (1971). An extensive literature on the subject does not mean that the problem has been solved. Attempts to find out so-called "best" solution culture of algae have resulted in the conclusion that probably there is no such solution. Algae can grow in a wide variety of culture media and their chemical composition and growth rate depend upon the environmental conditions, nutrients' availability, cell age, and species. Luxurious uptake when nutrients are incorporated in protoplasm at levels greater than those necessary for growth, and super luxurious uptake when some nutrients are stored rather than converted into algal protoplasm further complicate the problem of finding out the optimum nutrients concentration for algae. The uptake of nutrients is not necessarily correlated with actual metabolic requirements, and thus the metabolic rates and chemical

composition may not give any clue for nutrient requirement of algae.

The nutrient elements used by various workers for growing algae differ widely in quantity and ratio. It seems that the concentration in most cases was decided arbitrarily. Table 4.2.1 gives the amount of N and P, and their ratio used by different workers.

Many algae grow well in dilute mineral solutions containing soil extract. In Provasoli's(1956) words, "it is not surprising that soil extract has been such a panacea for growing exacting algae since it contains ammonia, thiamine and B_{12}", and that, "it might be expected that soil with an abundant and varied microflora will give extracts rich in all trace metals chelated by humic acid". Soil extract can be prepared by adding one part of garden soil to two parts water and steaming in an autoclave for one hour on two consecutive days, or the soil can be sterilized separately and boiled in water to give soil extract. The use of soil extract in mass culture of algae may be useful and has to be developed. The chelating agents, organic matter of soil and other elements not only can reduce the cost of nutrients, but may also give better growth.

2.4. CARBON DIOXIDE

Carbon budget in algal cultures is regarded as the most serious and difficult problem. The utilization of carbon

dioxide from water for photosynthesis by algae may disturb CO_2 - HCO_3^- - CO_3^{--} balance resulting in change in pH. This change (rise) in pH can affect the algal growth in number of ways. These include 1) change in carbon species, 2) solubility of nutrients and 3) metabolic changes due to pH.

The reports in the literature on the concentration of carbon dioxide needed for radiation saturated photosynthesis and utilization of bicarbonate by algae are conflicting. There are reports in the literature that algae can grow in a very high carbon dioxide concentration environment, at the same time Österlind (1943) has reported the retarding effects of high concentration of carbon dioxide and carbonate ions on the growth of algae. Carbon dioxide concentrations reported in the literature for algal cultures are generally in terms of its concentration in gas mixture with which cultures were assumed to be in equilibrium. Semenenko et al., (1966) have reported that reaching the equilibrium is very difficult even at very high rate of air gas mixture and these values may be misleading.

The cheap supply of fairly pure carbon dioxide is one of the decisive problems of algal cultivation. Carbon dioxide from a fermentation process seems to be the most appropriate source. The possibilities of using carbon dioxide from natural wells, from burning various fuels, calcination of lime stone, and other chemical processes have to be analysed. The cost of carbon dioxide can be reduced

to almost zero by combining the algal plant with the carbon dioxide production point.

2.5. OXYGEN

The inhibitory effect of oxygen on oxygen uptake and carbon dioxide fixation is extensively investigated (Turner and Brittain 1962, Myers 1964, and Gibbs 1970), but not adequately explained. The effect commonly known as Warburg effect is ignored in mass cultivation. Warburg effect is pronounced at high light intensity and low carbon dioxide concentration. In most of the mass cultivation units such is the situation, and there may be 30-40% inhibition due to this effect (Gibbs, 1970).

Degasification of the culture to liberate all the extra oxygen in the liquid culture would remove carbon dioxide dissolved in liquid, required for photosynthesis also. Degasification of the culture medium, and then saturation with carbon dioxide, may be a practical solution.

2.6. PHASE CONTACTING

The most widely used method for phase contacting (liquid-gas) is bubbling or sparging technique. In this method carbon dioxide-enriched air is forced through small orifices or tubes in the bottom of the culture vessel. Stengel (1976) has reported that use of pure carbon dioxide is as efficient as carbon dioxide-air mixture, rather the use of pure carbon dioxide is economical due to better control of pure gas flow,

and high residence time of bubbles in the culture medium due to higher density of carbon dioxide. The various phase contacting equipments are reviewed in Chapter 4.

2.7. MIXING

Mixing in algal cultures keeps the distribution of algal cells, the concentration of nutrients and mean irradiation per cell uniform. Mixing enhances the metabolic rates by replacing the stagnant film deficient in nutrients by fresh nutrient medium film (Soeder 1974). The degree of mixing required for each factor listed above is different, being lowest for uniform algal and nutrient distribution to maximum for desired high frequency intermittency. The rate and efficiency of photosynthesis can be increased by flashing light effect. This flashing light effect can be obtained in dense algal cultures by means of appropriate turbulence. Inspite of the sizable work done on flashing light pattern, there are uncertainties concerning appropriate length of light and dark intervals to match the light-dark reactions. The times reported in literature are of the order of 10^{-3} to 10^{-2} second (Emerson and Arnold 1933), 4×10^{-3} second light and 10^{-1} second dark (Phillips and Myers, 1953), and still higher are reported by Burk et al.,(1951). Another type of pattern uses equal length of light and dark period of 1 to 5 flashes per second (Rabinowitch 1956). As can be seen that on the basis of information available in the literature it is difficult to decide the desirable pattern of intermittency.

The random nature of turbulence causes another hindrance in utilizing optimum intermittancy. Fredrickson et al., (1961) and Powell, et al., (1965) have pointed out that it is possible to subject all the cells to a definite light dark pattern of same mean periodicity, but this would have very large variance from the mean value.

The gain in photosynthesis rate and efficiency by utilizing random turbulence is often questioned. This problem is theoritically and experimentally analysed by many workers. Fredrickson et al., (1961) used Lumery-Rieske mechanism (1959), and derived equations for the rates of photosynthesis in rectangular unstirred and rectangular fully agitated growth chambers. They found that optimum efficiency for the two cases do not differ, however, the rate in stirred chamber was considerably higher. Fredrickson and Tsuchiya (1969) have discussed and analysed two flaws in their assumptions in deriving above results. Miller (1962, 1963) using Lumry-Rieske mechanism calculated increase in photosynthetic efficiency due to mixing in Taylor apparatus. Experimentally an increase in efficiency was realized but this could not approach to the theoritically predicted value. Powell et al., (1965) considered the algal cultures to be in turbulent motion in a flat channel illuminated from both sides, and assumed turbulent velocity fluctuations normal to the direction of the flow. They modelled the motion of algae as a ten state Markov process, and used correlation between coefficients of

the Markov transition probability matrix and Prandtdl's mixing length theory of turbulent transport. Their calculations showed that the variance of transit time would be very large. In Powell's words "Turbulence alone is a poor mechanism for producing optimum light dark pattern." Gordon (1972) used similar approach to analyse Taylor apparatus. Gordon (1972) used three state Markov process to calculate rates and efficiencies, but did not relate the Markov Stochastic Matrix to the hydrodynamics. Seth (1974) simulated the photosynthesis by algae in turbulent channel flow, and using different models for algal motion and two different kinetic rate mechanism viz., Lumry Rieske (1959) and Tamiya (1949), showed that turbulent mixing in channel flow can achieve an increase in the rates and efficiencies of photosynthesis through intermittent effect due to mixing. He showed that superimposing random motion in a Taylor apparatus does not lead to any significant improvement. Fredrickson and Tsuchiya (1969) in a paper which is an excellent treatment of the subject have pointed out " - that intermittency effects can be utilized to enhance the performance of optically dense cultures--. ---, but whether this can be done without prohibitive expenditure of energy for agitations is still not perfectly clear". Fredrickson (1961), Powell (1965), Gordon (1972) and Seth (1974) have analysed the rate and efficiency of photosynthesis, but have not touched this important but unanswered question.

2.8 MOTION OF ALGAL CELL IN TURBULENT CHANNEL

The analysis of motion of algal cells in turbulent channels is required to calculate mean irradiation and in designing of algal tanks to obtain uniform mixing. An exact description of hydrodynamics in turbulent channel is an extremely difficult task. Navier-Stokes equation assumed valid in turbulent flow is never solved for active dispersent. The turbulent flow in rectangular channel has been studied, both theoritically and experimentally. Most turbulent measurements are derived by assuming statistical quantities describing the 'structure' as gaussion process, and in Eulerian frame, while most of the times analysis in Lagrangian measurement is required. Although Eulerian correlation coefficients can be related to the Lagrangian correlation function for short time, but velocities of the algae which are not identical to the fluid velocities make the problem further complicated.

An extensive work has been done on the measurement and prediction of longitudinal and transverse mixing coefficients in rivers to predict the mixing of waste discharged to water bodies and rivers and their purifying capacities. Most of these relationships between hydrodynamics and mixing coefficients are for passive dispersant, i.e., dispersant which are miscible and do not alter the velocity of the fluid. So this analysis cannot be used to predict mixing of algae.

2.9. ALGAL KINETICS

The exact description of algal kinetics is an extremely difficult task. Some of the complications involved are discussed in this section. Algal growth can be considered as made of two processes: photosynthesis and synthesis of cell material. Photosynthesis depends upon nutrients concentration, carbon dioxide concentration and light intensity. The rate and photosynthetic efficiency are affected not only by absolute values of nutrient components, but by their ratio, and mode of application also. Temperature of the culture, saturation light intensity and carbon dioxide effects are interrelated, and give nonlinear differential rate equations of higher order. Autoregulators present in algae make the problem further complicated. Photosynthesis is represented in terms of trapping centers (e.g., Chlorophyll) and their concentration keeps on changing with stages of algae. Mixing in algal culture affects the growth by making the algae and nutrients concentration uniform, changing the mass transfer rates, and exposing the cells to random intermittent light-dark pattern. High frequency intermittent light-dark pattern affects the growth and metabolic rates by flashing light effect, while low frequency intermittent pattern affects the algal systems through synchronization. Kinetic study of algae in agitated systems thus involves solving transport and growth equations simultaneously. The exact description and solution of transport equations itself is extremely complicated.

The kinetics of algal growth has been studied extensively. Many assumptions are made in order to make the analysis simple, and various models from exponential growth to models involving mixing and growth are reported in the literature. The series reaction mechanism presented by Lumry and Rieskey (1959) and Tamiya (1949) on the basis of the reaction velocity of Hill reaction in isolated chloroplast and intact algae are widely used. Tamiya (1949) described a model for the photosynthesis by algae in carbon-dioxide saturated environment by a set of reactions involving photosynthetic sensitizer and enzyme. The former accepts the energy from activated sensitizer to become activated enzyme. Tamiya et al., (1953) used a relationship similar to Monod model for light as limiting substrate, and have derived expressions for growth in optically thin and thick cultures. The photosynthesis can be modelled as a function of energy trapping centre concentration or mean light intensity or both. Tamiya et al., (1964) used cell mass for pigment concentration in their model. Fleischer (1935) found rate of photosynthesis to be proportional to the chlorophyll concentration. Bongers (1958) although did not name chlorophyll but reported that photosynthetic activity is constant when related to some photosynthetically active component. Sorokin (1960) and Nihei et al., (1956) supported the use of chlorophyll for modelling photosynthesis. Smirnov (1964), and Pipes and Koutsoyannis (1954) used light intensity in their models. Rabe and Benoit (1962) used mean effective light intensity and

mass concentration of algae to describe algal photosynthesis. Uren and Harsman (1971) used Lumry-Riesko mechanism to predict apparent growth rates of algae, and concluded that total chlorophyll concentration is a more adequate index of the trapping center concentration in above mechanism. Fredrickson et al., (1961), Miller, (1962, 1963), Powell (1965), Gordon (1972), and Seth (1974) have used simple algal kinetics, and solved these equations to calculate photosynthetic efficiency with different mixing patterns. These have been discussed in section (2.7).

2.10. REACTOR DESIGN

The algal reactors can be divided into two types; i) open systems and ii) closed systems. Closed systems are preferred for chemotrophic production of algae, in gas exchangers and in research where control of environmental conditions is important. Loss of carbon dioxide and water are the disadvantages, while cooling of the culture by evaporation and ease of construction and maintenance are the advantages of open systems. Open systems suffer from sudden unfavourable environmental conditions and spontaneous infections such as rotifers, mosquito, amoebae, etc.

The above two systems can be classified according to the phase in which algae grow e.g., solid phase reactors and liquid phase reactors. Different type of liquid phase reactors are reported in literature, such as: horizontal

tube culture (Fisher, 1956), vertical tube culture (Hindak, 1970), chamber culture (Mathern, et al., 1964), green house type culture (Watanabe, 1959), cuvette culture (Miller 1962), pond culture (Morimura et al., 1955 and ~~Swaldwood~~ Golueke, Oswald 1968 a), helical tube culture (Kaul, 1971) and wetted wall column (Sharma, 1969). Solid phase reactors are simple, easy to maintain and very easy to harvest. Sand bed solid phase reactors (Swaminathan, 1971) have the advantage that these can be used as a dewatering and sand bed drying units. The work done on solid phase reactor is scarce. Reid (1961) used wood, concrete and corrugated metal for algal cultivation. Swaminathan (1971), Singh (1972), and Joseph and Thakur (1970) used sand bed reactor, and their work is discussed in section 2.12. Continuous reactors of algal cultivation are reviewed by Retovsky (1966).

2.11. HARVESTING

Harvesting of algae is difficult due to its small physical size, negligible difference in density, and very low concentration. There are several methods of harvesting, and choice would depend upon the end use of the product. The work of Golueke and Oswald (1965, ~~1974~~) is an excellent treatment of harvesting the sewage-grown algae. Environmental protection agency (1971) and IIT, Kanpur (Sinha, 1969; Tikhe 1969; Ramachandran, 1971; and Sahni, 1973) have tried different methods of harvesting sewage-grown algae. Some of the important methods used for harvesting the algae are:

1. Centrifugation
2. Flocculation
3. Filtration
4. Flotation

2.11.1. CENTRIFUGATION - Centrifugation is a simple and continuous method of harvesting, which gives product of high quality and devoid of additive agents. There are some contrary views in the literature (Burlew 1953; Gotaas and Golueke 1957; Levin et al., 1962; ~~Oswald and~~ Oswald, Golueke, 1968) on the use of centrifuge. The objections are high operating and equipment cost. Setlak et al., (1970) have analysed the advantages and disadvantages, and have shown that cost and power are not prohibitive. They are of the opinion that centrifuge is the most suitable method of harvesting the algae without changing the chemical nature of the clear liquid from the centrifuge, and physiology of the algae.

2.11.2. FLOCCULATION - Removal by chemical flocculation involves the addition of reagents such as alum, lime, organic cationic flocculants, etc. Oswald (1963), and Environmental Protection Agency (1971) have used different flocculents and have reported cost analysis. The disadvantages of chemical flocculation are that algae and effluent contain lime, and high pH of effluent makes it undesirable for reuse and direct discharge into the river or other bodies of water.

Autoflocculation, a natural process of clumping algae cells together occurs when the temperature and pH of the culture are high. This natural phenomenon can be used as a means

of harvesting the algae.

Different theories have been put forward to explain the mechanism of bioflocculation of bacteria (Sahni, 1973); Zoogloearamigera theory,(Butterfield 1935); PHB (polybetahydroxy butyric acid theory (Crabtree, 1963), extra-cellular polysaccharides theory (Mckinney 1952; and Pavoni et al., 1972). According to Pavoni et al., (1972) bioflocculation is the result of interaction of naturally produced high molecular weight long chain poly electrolytes with organisms to form an aggregate.

Induced flocculation is the phenomena which uses the naturally occurring but mechanically induced polyelectrolytes to form algal aggregates. Induced flocculation may be an economical method of preconditioning the algae before harvesting by sedimentation. The work done on this aspect at Kanpur is discussed in section 2.12.

2.11.3. FILTRATION - The use of filter papers, fine mesh metal, nylon, cotton, and woolen screen of wide varieties in filtering algae is reported in the literature (Sahani, 1973; Oswald, 1968; and Land, 1969). The work done on sand filters is reported by Brown (1969), Folkman et al., (1971); Glyoyna et al., (1955), and Environmental Protection Agency (1971). The studies on electrokinetic phenomena in the filteration of algae, Zeta Potential, and adhesion of algae are reported by Foess et al., (1969), Hegewal (1972) and Nordin (1967) respectively.

2.11.4. FLOTATION - Golueke and Oswald (1965) used different floatation reagents, and found most of them ineffective except two. Ramachandran (1971) and Kumar Sastry (1972) used foam flotation for harvesting algae in batch and continuous processes using hexadecapyridinium chloride ($C_{21}H_{38}NCl$). Levin et al., (1962)have reported the effect of pH, aeration rate, aerator porosity and feed concentration on harvesting of algae by flotation.

2.11.5. DRYING - Drum drying, sun drying and spray drying are the possible methods of drying the algae to reduce the moisture content of algae to a level (6-8%) at which the product can be stored safely. Drum drying is a rapid process, and losses in nutritive value of algae in this process are expected to be low. Sun drying of algae is economical but slow. A drying area equal to 5% of growing area may be sufficient for sun drying (~~Oswald and~~ Golueke & Oswald, 1968). Properly designed sand beds can be used for dewatering and dehydration of algae. The drawback of this method is that algae is contaminated with sand. Sun dried algae could transmit agents of disease and thus should be sterilized before use. Kapoor (1970) analysed the solar dryer for agricultural products and calculated optimum tilt of the surface of drying. The effects of drying methods on the nutrient contents of algae are reported by Subulakshmi, et al., (1976) and Pabst, et al., (1964).

2.12. WORK DONE IN INDIA

The work on mass cultivation of algae with engineering approach was started at Indian Institute of Technology, Kanpur in 1968 (Leader, Dr. Seshadri, C.V.). At Indian Agricultural Research Institute, Dr. Venkataraman's group have been working on effect of algal inoculation in paddy fields and growing algae over thin layers of sterilized soil in shallow trays to inoculate paddy fields. Dr. R.D. Fox of Laboratorie de la Roquette, France, made a tank viz., respirating basin for Spirulina at Navsari, Gujarat, in 1972. The work on growing Scenedesmus acutus for food was started at CFTRI, Mysore, in 1973 with German Government cooperation (Dr. W. Becker, Leader). This section reviews the work done at different centers on mass cultivation of algae in brief and the work done at Kanpur in detail.

a) WORK DONE AT KANPUR

The work on designing the algal reactors was initiated by Prof. Seshadri in 1968 at Indian Institute of Technology, Kanpur. Various reactors were designed, fabricated and tested. The reactors were named CPRA (Continuous Photosynthetic Reactor for Algae).

At Kanpur the approach of bioregenerative systems was adopted, i.e., finding out the conditions for maximum efficiency, identifying the critical points and then investigating the possibilities of reducing the cost by research and development through engineering approach.

To find out maximum efficiency and identify critical points, closed systems were choosen. The limitations of closed systems i.e., high energy requirement, and necessity of a cooling system were known to them, but better control, low water and carbon dioxide losses, and possibility of using three dimensional structure for absorbing the radiations were some of the advantages of closed system for the choice.

Higher photosynthetic efficiency is one of the most important advantage of algal systems. Thus the design of the reactor demands that saturation losses be avoided to achieve maximum efficiency. The use of intermittent light effect induced by mutual shading of algal cells by turbulence is one possible solution. However, the efficiency of such a system is many times questioned. Another way of avoiding saturation losses may be by extending radiation absorbing surface in the direction perpendicular to the falling radiations. A design to satisfy this requirement in outdoor culture may be highly complex due to the variable character of solar radiations. A simplified model using the above phenomenon is the spray columns. Water spray may be designed to give the structure of most efficient crop e.g., sugar cane plant.

Khanna and Kohli (1967) and Sharma (1967) used the above idea and designed CPRA Mark I and Mark II. CPRA Mark I used the principle of wetted wall column for photosynthetic reactor. In CPRA Mark II, Sharma provided a rough surface for the growth of algae and made provisions for introducing

carbon dioxide and carbon dioxide air misture. However, no conclusive information regarding the growth of algae was given.

Kaul (1969) designed a continuous helical coil reactor viz., CPRA Mark III. He envisaged the increase in efficiency of light utilization through intermittency due to secondary vortices formed in helical coil.

Fluid particles flowing in curved channel under the axial pressure gradient, instead of flowing linearly as in the case of a straight channel, moves away or towards the center of curvature of the channel, depending upon the normal reaction at the containing wall. Due to these disturbances, a secondary flow is set up in which the fluid near the top and bottom moves inwards and the fluid in the middle moves outwards. This circulatory flow in the form of free vortex is called secondary vortices and is superimposed on the main axial flow. Shaukat and Seshadri (1973) have made pressure drop measurements in Archimedian spiral coils of circular cross section. Shaukat (1974) solved transport equations applied to fully developed flow in curved channels using approximate analytical method of weighted residuals.

The above idea of secondary flow in curved coils was used in designing CPRA Mark III. This reactor consisted of two reactors: 1) photosynthetic reactor (PR) for energy absorption and 2) formative metabolic reactor (FMR) for cell division and

other processes. PR was a helical tube of 3" coil diameter, 3/4" tube diameter, having 15 turns of average pitch 1", fitted with nine 40 watt fluorescent tubes for energy. FMR was a vertical glass column 3" dia 5 ft. long, with the provision of gas, and nutrient media outlets, inlets and sampling port. Nutrient medium flowed continuously in to FMR and was pumped back to PR. Chlorella from IARI, New Delhi, India was used.

CPRA Mark III was a continuous reactor, and with all the advantages of continuous reactors over batch reactors was best suited for studying the effect of turbulence on the growth. The advantages of Kaul's reactor, CPRA Mark III over Miller's reactor (Miller 1962) using vortices generated in an annular space by revolving the inner cylinder were that Mark III was a continuous reactor, and it was possible to separate and control the dark and light reactions in the two reactors viz., FMR and PR. Thus cultures of Kaul were subjected to high frequency intermittent light and dark pattern in PR, and low frequency light and dark pattern in PR and FMR. Probably this was the first time when the effect of simultaneous high frequency and low frequency intermittent pattern on growth were realised. High frequency intermittent pattern improves efficiency by flashing light effect, while low frequency intermittent pattern works through synchronization. Kaul's reactor used turbulence created by secondary flow to achieve intermittency, and till this time there was no clear cut idea, that whether random turbulence can improve the growth. This was later analyzed by Fredrickson et al. (1969) and Seth (1974).

The main drawback of the CPRA Mark III was the adhesion of algae on the wall of the surface during experiment and shading the algal cells.

Kaul (1969) found that increase in illumination from 5 fluorescent tubes to 9 fluorescent tubes did not change the growth rate, and this was probably due to the lower algae concentration (.25 g/l). Intermittency effect can be realized only in dense culture when all the light is absorbed in 1/10 of the total depth (Fredricken 1969). One important observation made by Kaul (1969) was that concentration of algae at steady state decreased with decrease in holding time, but dry weight obtained (algae concentration at steady state x flow rate) showed a maxima at flow rate of 720 cc/day. Kaul (1969) found that steady state concentration is significantly changed by growing algae in batch for 36 hr before starting the continuous operation. The change in steady state concentration by changing initial condition is possible due to the interaction of toxic substances and growth promoting substances produced during growth (Misra, 1971).

Sharma and Lal (1967) used CPRA Mark III. They used bottled carbon dioxide (from Indian Oxygen Limited, Kanpur) and air mixture. The growth of algae was reduced in presence of carbondioxide, possibly due to some toxic substances present in carbon dioxide. However, no attempts were made to find out the toxic substance or purity of the gas. Mittal, Singh and Kirty (1969) used mixed algal culture from IIT, Kanpur

oxidation pond. They studied the effect of mono-ethanolamine, MEA as absorber of carbon dioxide on the growth of algae. They used two flasks to grow and compare the growth of algae under identical conditions. They found that MEA reduces the growth rate of algae. The possible explanation given was that MEA absorbed all the carbondioxide and does not release it for algal growth at low temperature. In an other set, Mittal et al., (1969), used CPRA Mark III with helical tube reactor, PR in vertical position, but this did not give any improvement.

Mittal et al., (1969) used rotary drum filter to grow algae on the surface of drum. The advantages of drum filter claimed were: easy to harvest algae, and easy control on light dark pattern by adjusting the speed of the drum (Biological discs of asbestose or light material, corrugated PVC sheets are used for biological waste treatment (Khan and Siddiqui, 1972). Mittal et al., (1969) designed a carbon dioxide generator using kerosene and calculated that 0.19 liter/hr kerosene would give 239 l/hr carbon dioxide at NTP, and 1610 Kcal/hr. This heat produced can be used for drying algae, and this can be shown that with this arrangement algal systems would require very little extra energy.

At this stage it was realised that turbulence to achieve high frequency intermittent pattern would require very high energy, and the use of random turbulence to improve the photosynthetic efficiency was questioned. The analysis of Fredrickson (1969) lateron proved that the improvement in

efficiency by random turbulence is very doubtful. At this stage the closed system reactor with secondary vortecies was abandoned, and the work on open systems was started.

Open systems with rough surfaces were designed. Simplicity of the reactor and ease of harvesting are the advantages claimed for solid-bed reactors. Swaminathan (1971), and Joseph and Thakur (1971) used a thermocole bed arranged at an agle of 60°. Nutrient medium was allowed to flow down the slope by using a pump and a 200 watt bulb suspended over the bed was used as a light source. It was found that medium does not flow uniformly over the bed, and there was no significant growth. At the same time sand bed reactor was designed. The irregular surface of sand bed and filtration action of bed due to minute pores were the advantages claimed. On testing they found that it is difficult to solve the problems of non-uniform flow of nutrient medium, erosion of sand, and channeling in small bench size reactor. They tried different distributor systems without success.

In another design, the sand bed was kept horizontal, and holes were made in the bottom of the bed to collect and recycle the filterate. This bed reactor was found successful. Another solid bed reactor, CPRA Mark IV was fabricated, which used a circular bed of gravels over which a thin layer of sand was laid. The filterate was collected from center of the bottom and was pumped back to the reactor. Provision was made to supply carbon dioxide or carbon dioxide and air

mixture near the over flow weir. Joseph and Thakur (1971) used this reactor and studied the effect of light intensity, time and bed depth on the growth of algae. A factorial design of experiments was carried out, and multiple regression analysis gave a relationship between yield of algae and the above variables. Joseph and Thakur (1971) showed that log fit and exponential fit are better than ordinary fit. They found that exponential fit was marginally better than log fit, but excluded the important variable, illumination in final equation. Time of growth and illumination were found significant variables, while bed depth was insignificant variable. The regression equation was:

$$\ln Y = -11.12 + .56 \ln X_1 + 1.22 \ln X_2 + .31 \ln X_3 \quad .. (2.1)$$

where, Y = Yield of algae (decigram/liter)

X_1 = Light intensity in watts

X_2 = Time in days

X_3 = Bed depth in cms.

Swaminathan (1971) used randomized fractional factorial design to obtain the effect of inoculum concentration, liquid depth, light/dark ratio, and carbon dioxide flow rate on the growth of algae using CPRA Mark IV. Swaminathan (1971) reported that log fit is better than ordinary fit. Swaminathan (1971) obtained regression analysis equation:

$$\ln Y = -19.89295 + .858 \ln X_1 + .8 \ln X_2 + 1.27 \ln X_3 \quad .. (2.2)$$

Where, Y = Growth in g/m^2

X_1 = Inoculum concentration mg/l

X_2 = Liquid level in cm

X_3 = L/D ratio

In these experiments the surface of response was not determined, and this was probably due to the limited number of runs and narrow range of experimentation.

Singh (1972) used a closed algal system using sand bed for the growth of algae. His reactor, CPRA Mark V consisted of seven solid sand beds arranged one over the other with the help of a stand. Each bed was a circular wire mesh (2 ft dia) covered with a filter cloth and a sand bed over it. Light was provided by ten fluorescent tubes fixed to a cylindrical drum which also worked as a reflector. On the upper end it had a fixed sparger to spray the nutrient media in dispersed phase using 4.5% carbon dioxide and air mixture. In this way, Singh (1972) extended the light absorbing surface vertically, perpendicular to the direction of radiation. It was expected that this bed in series would separate the cells according tothe size, and synchronize the culture to give better growth. The experiments were carried out several times but there was no significant growth. The possible reason given was that the transfer of nutrients to cell was not enough because cells were not submerged in the nutrient media. Swaminathan (1971) has shown that depth of liquid over the bed is a significant variable.

Singh (1972) used a rectangular sand bed reactor to find out the degree of growth in liquid and on solid phase. Although it is difficult to separate the effects of shading, filteration rate, and transfer of cells from liquid to solid and vise versa to conclude, but Singh observed that growth of algae on solid is more than in liquid phase.

Misra (1975) used a inclined cascaded reactor. This cascaded reactor was equivalent to a series of stirred tank reactor, and had advantage of smaller variance of residence time in each reactor. It was expected that this would give synchronization of algal cells, and thus better growth (Fredrickson, 1969). This reactor was tested and it was found that there is a lot of mixing in first three cascades and last three cascades were almost unmixed. The algae settled down in last two cascades and thus there was no recycling of algae. The design was hydrodynamically unsatisfactory and was abandoned.

Misra (1975) cultivated Spirulina (from Laboratori dela Roquette, France) indoors and outdoors. It was found that Spirulina grow in indoor cultures but was bleached in outdoor cultures. Cultures were covered with a cloth to reduce light, and then some growth was observed. The effect of adding glucose on the growth of algae was examined and it was found that glucose increases the growth of Spirulina significantly.

Seth (1972) analysed the effect of turbulence on the

growth of algae in turbulent flows. He used kinetic models of Lumry and Rieske (1959) and Tamiya (1949) in his analysis. Seth (1972) concluded that superimposition of random turbulence may lead to some improvement in efficiency but superimposition of random turbulence on secondary vortecies in Taylor apparatus does not lead to any improvement in efficiency.

b) WORK AT KANPUR (DEPARTMENT OF CIVIL ENGINEERING)

As stated earlier the algae play an important role in waste treatment. The work done at Environmental Engineering Division, Department of Civil Engineering, IIT, Kanpur is reviewed in this section.

Bokil (1967) studied the effect of stratification on the growth of algae in a laboratory scale oxidation pond model. Manmohan Rao (1969) used oxidation pond model to treat fertilizer waste rich in nitrogen. He found that growth of algal kinetics resembles that of bacteria, and that recirculation of pond effluent increases the yield of algae. Sinha (1969) studied the harvesting of algae from oxidation pond by using chemical flocculents and filtration. High doses of alum were required for flocculation. Chemical flocculation with alum at reduced pH of 7 along with $KMnO_4$ cost Rs.6.9/lb of dry algae. Clogging of filter and need of frequent back wash was observed in filtration studies. Tikhe (1969) used a variety of algicide and coagulents. He found that $CuSO_4$ is effective in controlling algae. $KMnO_4$ was found better than $CuSO_4$. Ferric chloride was found, as better coagulent

than alum. Chlorine and iodine were found less effective than $KMnO_4$. Ramachandran (1971) studied the harvesting of algae by foam flotation and examined the effect of surfactant concentration, air flow rate, pH and the effect of coagulation of algae with alum on flotation. Saini (1971) examined anaerobic digestion of algae to produce gas, and various harvesting methods. Saini (1971) found that algae harvested by auto-flocculation and foam flotation were suitable for digestion, while algae harvested by alum coagulation were unsuitable. Saini (1971) reported 51% destruction in volatile material in 30 days detention time at a loading rate of 0.03 lbs/cubic ft/day of volatile material fed. Sastry (1972) used cetyl pyridinium chloride and other indegeneous surfactant agents and examined the effects of air flow rate, surfactant concentration and detention time on stripping parameters.

Sahni (1973) induced flocculation by blending algae and recycling algal suspension through pump. Sahni (1973) claimed that in these methods the flocculation was induced by some high molecular weight polyelectrolites released by mechanical stresses, which agglomerate the algae. His results showed that algae can be recycled to increase its settleability. He found that recycle of blended algal suspension containing polyelectrolytes responsible for aggelomeration does not increase the COD of the algae effluent i.e., there is no increase in waste treatment plant load. There are reasons to say that this method may be economically feasible and needs

to be developed.

c) WORK AT OTHER CENTERS

R.D. Fox of Laboratoire de la Roquette, France designed and built a respirating basin to grow Spirulina for food. Culture basin was a shallow pond 11 m x 3 m x 0.06 meter deep, made of bricks morter and cement. A pull bar was used for stirring the culture. Growth rates from 3.75 g/m^2 to 7 g/m^2 were obtained. Fox calculated the cost of protein as Rs.5.80/kg, and proposed a 2 years programme for the cultivation of algae on large scale.

At Auroville, Pondicherry, India, algae are grown in circular tanks 21 feet in diameter and 24" in depth by using wind mill for mixing the algal cultures. They grow Chlorella, and use it in their diet.

Bhabha Atomic Research Center, Bombay, is experimenting on blue green algae Anabaena Torulose. Their emphasis is on mechanism of nitrogen fixation, physiology of induction of nitrogen fixing sites, and the availability of fixed nitrogen as extra-cellular product (Sharma, 1974).

Banaras Hindu University, Banaras, is working on algae and their emphasis is on the transfer of nitrogen fixation gene of algae to rice through a virus (Sharma, 1974).

Central Rice Research Institute, Cuttack, and Indian Agricultural Research Institute, New Delhi are working on blue green algae to use as biofertilizer.

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CHAPTER 3

MATERIALS AND METHODS

3.1. PLACE

The studies reported in this thesis were conducted at Central Food Technological Research Institute, Mysore, Karnataka, India. The experiments were performed inside the laboratory (indoor cultures) and outside the laboratory in open tanks.

In an experiment it was observed that sun set and sun rise at Mysore are very sharp. Figure 3.1 shows the solar radiation, culture temperature and air temperature of a day in December and May. It was found:

The time weighted average temperature of the day was at times 5 hours after sun rise, and 1.5 hour before sun set. The average temperature of the 24 hour-period was 4 hours after sun rise, and 1 hour after sun set. Maximum temperature of the day was between 2-3 P.M. In Mysore there was approximately 12 hr sun and 12 hours dark. Average radiation of the day was at 1 hour after sun rise and 1 hour before sun set.

3.2. STRAIN

Scenedesmus acutus 276-3a obtained from Kohlenstoff-biologische Forschungsanstalt, Dortmund, Federal Republic of Germany was used in all the experiments.

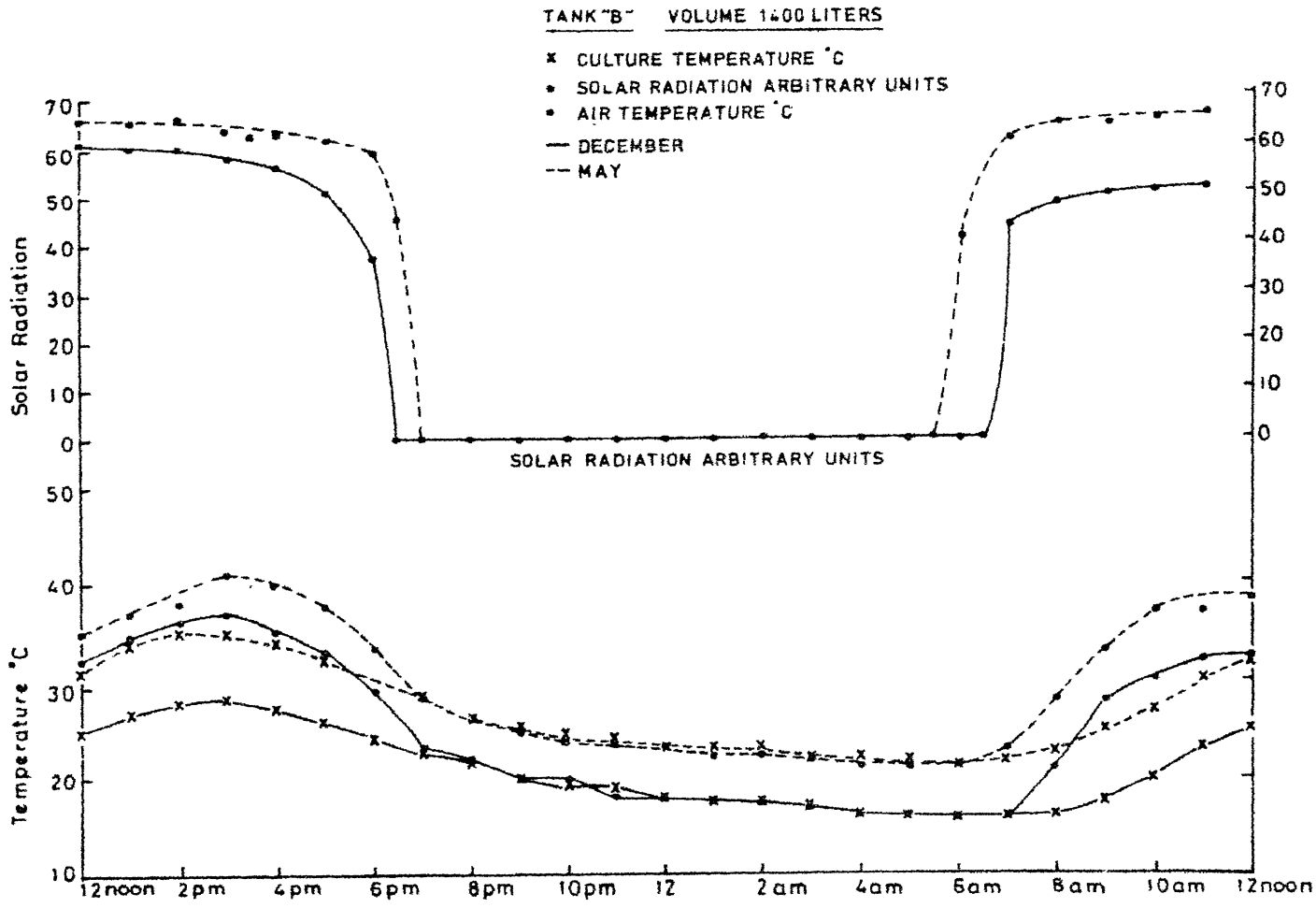


FIGURE 3.1 TEMPERATURE (OF THE AIR, CULTURE) AND SOLAR RADIATION AT DIFFERENT TIME OF THE DAY.

3.3. NUTRIENT MEDIA

Commercial urea 0.1 g/l as a source of nitrogen, and Scenedin 0.2 g/l as a source of P, Mg, Fe, S and other trace materials were used. The composition of Scenedin is given in Appendix 4.

3.4. WATER

Water from Mysore water supply was used in all the outdoor experiments without any treatment. Hardness, chlorine and iron ions were determined, and are given in Table 3.4.

TABLE 3.4

ANALYSIS OF WATER USED IN OUTDOOR CULTURES

Hardness	7° Hard; 1° Hard = 18 mg $CaCO_3$/1
Chlorine	less than 0.1 ppm
Iron total	less than 0.1 ppm
pH	8.3

3.5. INDOOR CULTURES

All the indoor experiments were performed in thermostats specially designed for algal cultures by Kniese Apparatebau GmbH Marburg (Figure 3.5). Cylindrical tubes, conical in shape at bottom, 28 cm long and 3 cm in diameter, closed by double bore silicone rubber stoppers were used. One tube which went down to the bottom of the culture tube was connected

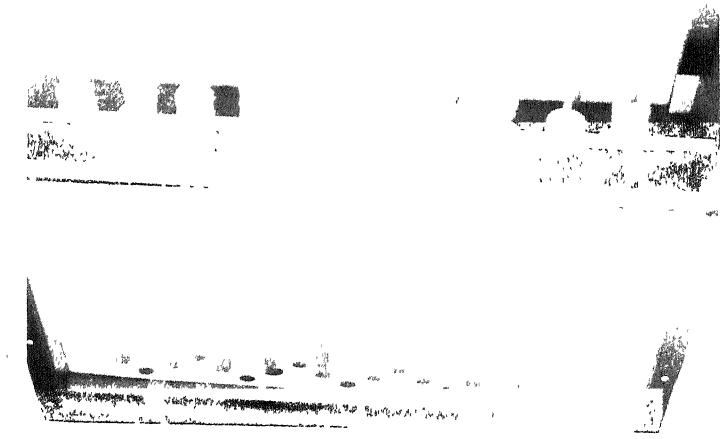


FIGURE 3·5 THERMOSTAT FOR INDOOR CULTIVATION

to air line through air filter and regulating valve (Figure 3.5). The air flow rate for 100 ml algal suspension was 300 - 400 ml/minute. This flow rate of air was enough to keep the algae and nutrient media in suspension. Each side of thermostat could take 10 tubes, and was illuminated by five 40 watt cool-white fluorescent tubes, which gave 6,000 to 7,000 lux light on the surface of the culture tubes. Timers were attached to each thermostat to program the light-dark duration by switching on and off the lights. The tubes were kept in the thermostat randomly, and their positions were changed daily to neutralize the effect of position of the tubes and aeration rate on the growth. Indoor culture studies were customarily conducted with 100 ml nutrient media (0.1 g/l Urea, 0.2 g/l Scenedin) in distilled water at 25-27°C, unless specifically mentioned. Absorbance and pH were measured at least once daily in the morning after making up the volume. Microscopic examination for contaminants was done at least every alternate day.

3.6. OUTDOOR CULTURES

The tanks of Indo-German Algal Project, CFTRI, Mysore were used. An overview of outdoor cultivation system of IGAP is shown in Figure 3.6.1. As shown in the figure there were 15 tanks (2A tanks, 8 B tanks and 5 C tanks of 55, 11 and 5 meter^2 surface area respectively). Figure 3.6.2 shows a closer view of an 'A' tank. These tanks were made of blue PVC Sheet. The dimensions of the tanks are given in Table 3.6.1

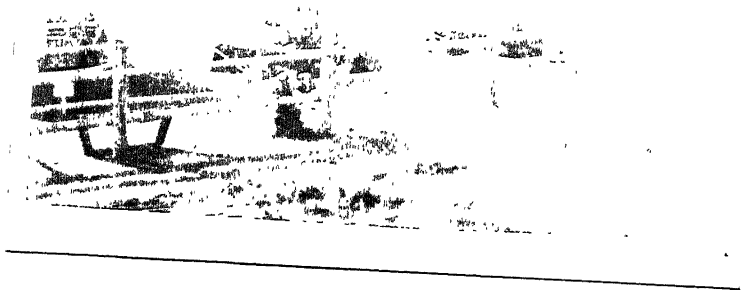


FIGURE 3·6·1 OVERVIEW OF IGAP OUTDOOR CULTIVATION SYSTEM

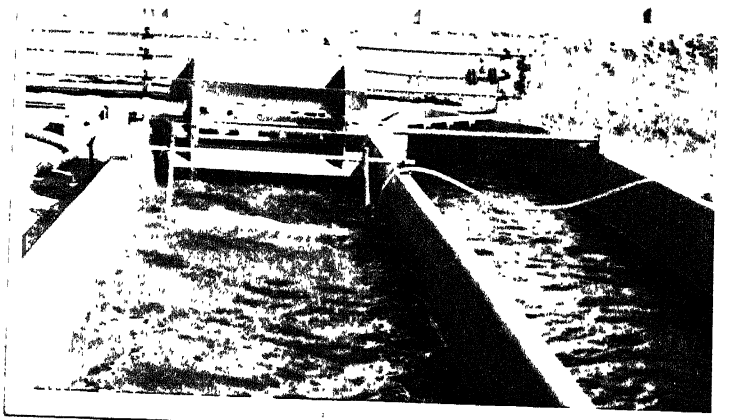


FIGURE 3·6·2 CLOSE UP VIEW OF AN 'A' TANK

and Figure 3.6.3. The turbulence in these tanks was provided by paddle wheels, driven by AC motors. Air from a blower was passed in the tanks through porous tubes from 8.30 A.M. to 5 P.M.

Normal operation of a tank begun with the removal of all algae from previous run. The known volume of water metered by water meter was taken in tank, urea and Scenedin were added, and was inoculated by a fresh culture of algae to give algae concentration from 0.05 g/1 to 0.1 g/1. Tanks were generally started in the evening to avoid loss of thin cultures by photooxidation. Evaporation losses were made up by adding water in the evening, and pH, absorbance and temperature were measured at least once daily in the morning. Tanks were swept before taking samples. A fine wire mesh was kept near the paddle wheel across the flow to collect and remove insects, leaves, etc. Bottled carbon dioxide was metered by rotameter, and bubbled through polyethylene pipe, which was kept lengthwise in the tank. A trnasparent polyethylene sheet 400 x 75 cm floating on the surface of the culture was used to entrap and increase the holding time of carbon dioxide bubbles.

TABLE 3.6.1

DIMENSIONS AND OTHER DETAILS OF THE TANKS

Tank	Length cm X	Width cm Y	Paddle Wheel size, cm b	d	Depth cm	Motor Kw
A	2270	108	106	22	40	0.37
B	292	54	56	22	28	0.18
C	290	54	56	22	28	0.18

3.7. HARVESTING

Harvesting laboratory of the IGAP was separate from the biological and chemical laboratory. Centrifuge, drum drier and blower used were in this laboratory.

a. CENTRIFUGE

Westfalia separator type SA 7-06-076 was used to centrifuge algal suspension from outdoor tanks. This centrifuge was disc type, and plate thickness was 0.05 cm. In this type of centrifuge, suspended solids accumulate on the side of bowl until they reach a predetermined level, and then are discharged as the bowl opens. The bowl closes and normal operation continues after the ejection of solids, which occurs in 5 to 15 seconds depending upon preset value. There was provision to direct the centrifuge effluent either to algal tanks or to effluent tank.

b. DRIER

Escherwyss drum drier model ESI.55 was used for drum drying the algae. The drum drier is heated up by three induction coils, and the temperature of the drum surface is maintained by controlling current in the coils by automatic controller. The algal slurry is sprayed on the surface of the drum by air ejection and rotating discs.

3.8. PHYSICAL ANALYSIS

3.8.1. OPTICAL DENSITY - The optical densities of samples were measured by using Medico Photometer tube of 2 cm in diameter at 560 nm. The algal samples were stirred before taking optical density by magnetic stirrer. Two to four samples were taken and mean of the optical density was recorded. The least count of the photometer is 0.005 between 0 and 0.3 optical density unit.

3.8.2. LIGHT - Illuminance was measured by using Lux meter (O, Lang, Berlin), or Joule meter (Yellow spring instrument Co, Ohio). Illuminance was measured in the morning between 8.30 to 9.30 A.M.

3.8.3. DISSOLVED OXYGEN - Dissolved oxygen in cultures was measured by dissolved oxygen meter (Model Oxy 56) WTW. Whenever required a Sevogara S recorder (Goerz Electric GmbH Wien) was used to record the dissolved oxygen. The electrode was polarised for 12-24 hours before use. The meter was standardised every third day with zero dissolved oxygen solution and air saturated water.

Dissolved Oxygen, pH, temperature of the culture, temperature of the air and solar radiations were measured and recorded on a six channel recorder for out door experiments. In addition, temperature of the air, and rain were recorded continuously.

CHAPTER 4

PHYSICO-CHEMICAL PROPERTIES AND GROSS PARAMETERS

This Chapter is divided into two sections: 1) Physico-chemical properties required for algal mass culture, and 2) The effect of gross parameters on the growth of algae. Physical properties of algal cultures like density, viscosity, packed cell volume, and light absorption coefficient were determined and are reported. Gross parameter section of this chapter deals with the effect of nutrient concentration, mixing and aeration, inoculum size, recycle of clear liquid from centrifuge to tanks, effect of pH, and performance of centrifuge and drier.

4.1. PHYSICO-CHEMICAL PROPERTIES

Physical properties of algal culture and algal slurry are important for the design of algal tanks, harvesting units, and drying units. Density and viscosity of algal suspension are required in solving flow, mixing, and mass transfer problems.

4.1.1. OPTICAL DENSITY

The relation between optical density and dry weight content varies according to the environmental conditions of algal culture, and from strain to strain. In this set of experiments the relationship between optical density and dry weight content for Scenedesmus acutus was determined.

a. METHOD

500 ml to 2 liter samples were taken from tank culture, and were filtered through coarse cloth to remove contaminants (insect, etc.). Optical density of sample was determined using Medico Photometer at 560 nm in 20 mm diameter Medico Photometer tube. Two methods were used for estimating dry weight content. Experiments were done in duplicate.

i) A known volume of sample was centrifuged at 3,000 RPM for 10 minutes. The sedimented cells were washed thrice in distilled water, transferred to weighing bottles, dried overnight in oven at 110°C, and weighed.

ii) In this method the algal suspension was filtered through a millipore filter of 3 μ pore size. The algal slurry on filter paper was washed thrice by distilled water, dried in oven at 90°C and weighed. The method of Algal Assay Procedure, Bottletest (1971) was used.

b. RESULTS

Dry weight contents in mg/l obtained in two methods are plotted against optical density in Figure 4.1.1 A. The dry weight contents using filter paper were always higher than centrifuge method. This means that some cells are lost during washing. Correlation coefficients by regression analysis computed from the data are 1.03 $\pm$ 0.085 for centrifugation method and 1.1 $\pm$ 0.06 for filter paper method.

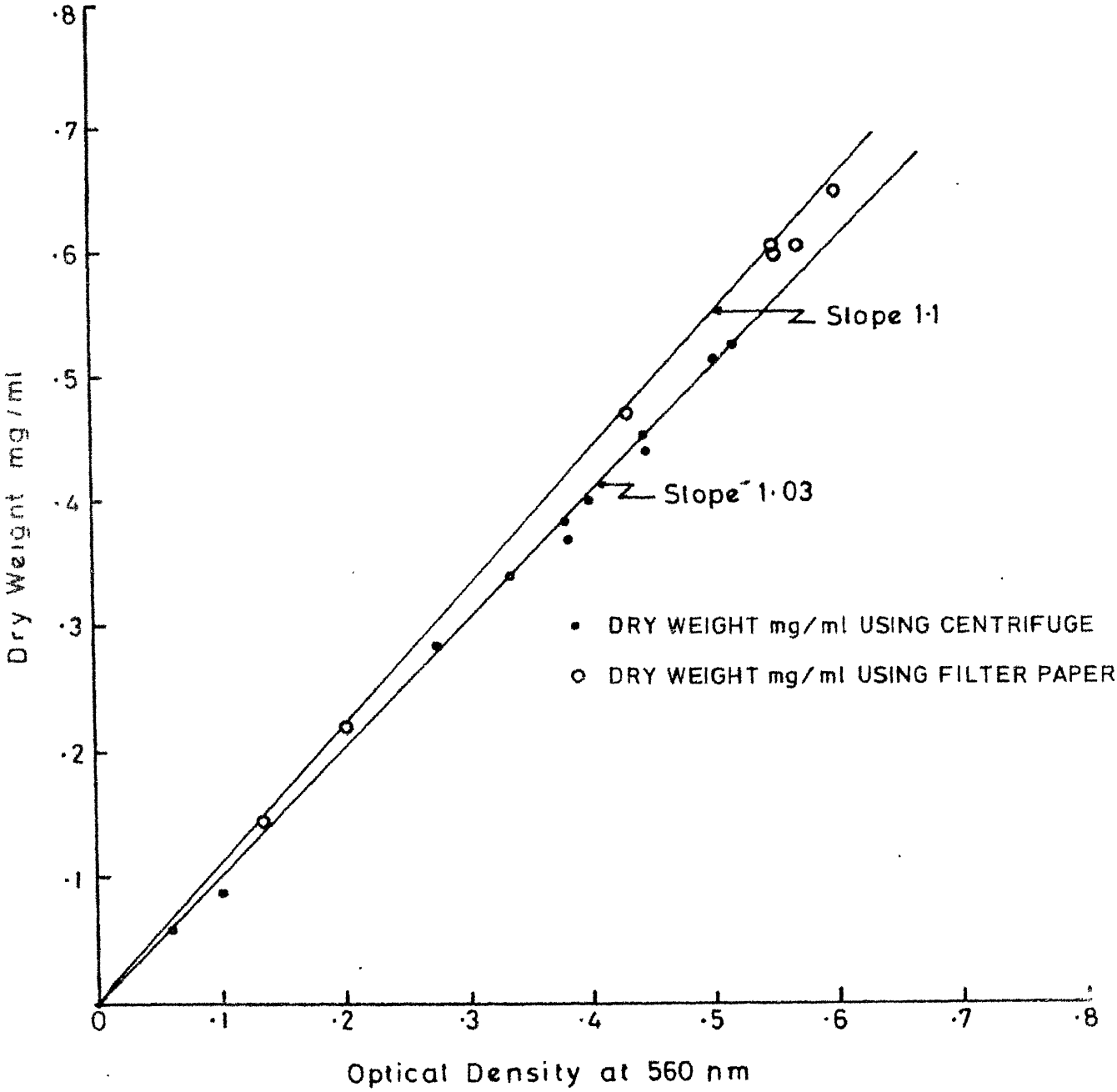


FIGURE 4·1·1 DRY WEIGHT VERSUS OPTICAL DENSITY

c. DISCUSSION

Different workers have used different wavelengths for optical density measurements. Algal Assay Procedure (1971) does not suggest any wave length for absorbance measurements. It is reported (Algal Assay Procedure 1971) that relative optical densities at 600, 680 and 750 nm are of the order of 70:500:1. In our experiments it was found that optical density at these wave lengths are of the order 5.4:6:1. A record of optical density at different wave length is shown in figure **4.1.1A.** In this work optical density at 560 nm was measured because the equipment with a filter of 560 nm was readily available, the relationship between dry weight and optical density was found linear at this wave length, and there was almost 1:1 relationship between optical density and dry weight. Zahradnik (1967) measured the optical density of Scenedesmus quadricauda, Chlorella pyrenoidosa and Chlorella ellipsiodea at 420, 560, 690 and 750 nm, and found that the values of optical density at 750 nm exhibit the lowest variance. It was found in our studies that the double beam spectrophotometer (model 124, Perkin Elmer) does not give stable optical density reading at 750-730 nm. In this thesis all graphs are reported in terms of optical density of culture. Numerically the value of grams/litre and optical density can be taken as almost the same.

4.1.2. DENSITY

Sinking velocity of algae, which is a function of density is an important parameter in designing settling tanks.

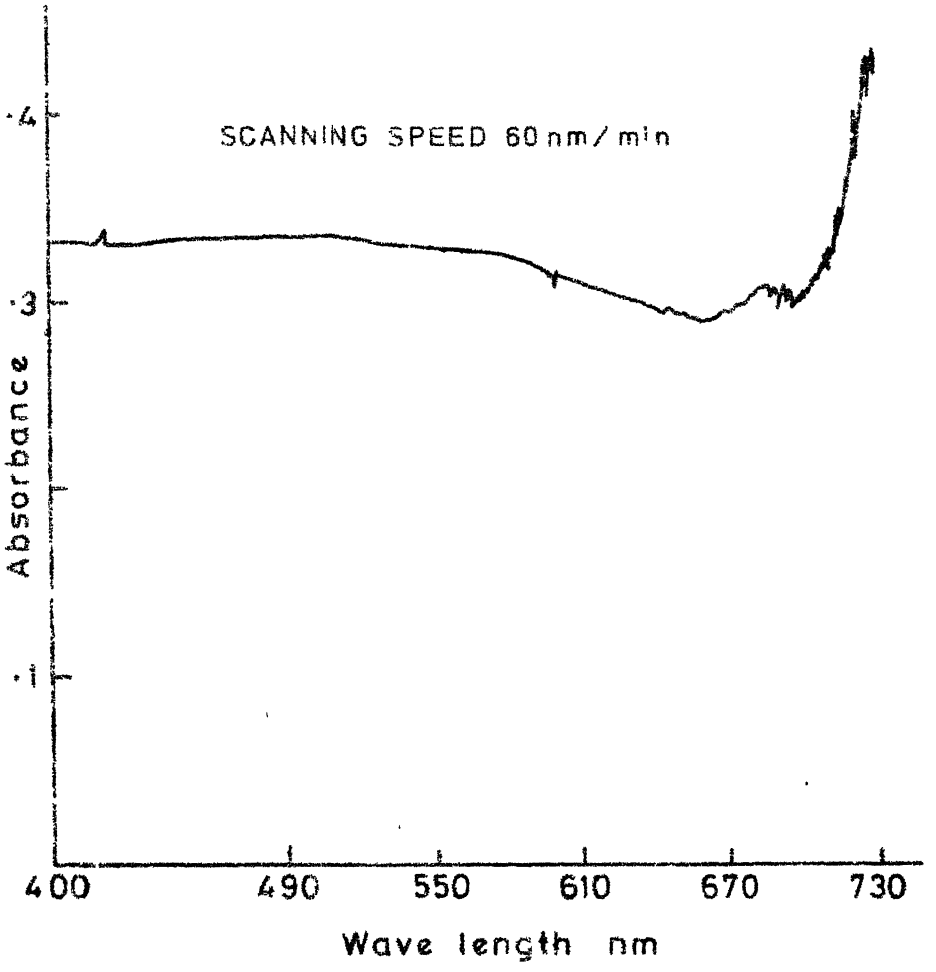


FIGURE 4·1·1·A ABSORBANCE OF ALGAL CULTURE AT DIFFERENT WAVE LENGTH

Bella (1970) developed one dimensional mathematical model to simulate the combined effects of sinking velocity and vertical mixing on the growth of algae in lakes. Algal sinking velocity and vertical mixing control the relative algal population in a mixed culture of algae. Increase in mixing always favours the algae with higher sinking velocities by exposing them to the sunlight in the euphotic zone, and reduction of euphotic zone depth favours the algae with lower sinking velocities (Bella 1970). Most of the blue green algae have lower sinking velocity and its growth rate in lakes can be reduced by artificial mixing.

a. METHOD

Relative density of algal suspensions of different concentrations were determined by using density bottles. All the densities were determined at 30 $\pm$ 0.5°C.

b. RESULTS

Relative density of thick algal suspension from centrifuge was determined and is shown in Figure 4.1.2. The density of algae versus dry weight is given by a line $\rho = 1 + .0033w$. Where ρ is relative density and w is % dry solids in the suspension. This relative density and dry weight relationship can be used to determine the dry weight per unit volume of slurry by relative density measurement using hydrometers.

4.1.3. WET PACKED CELL VOLUME

For thick algal suspensions, the optical density method is not suitable for determination of

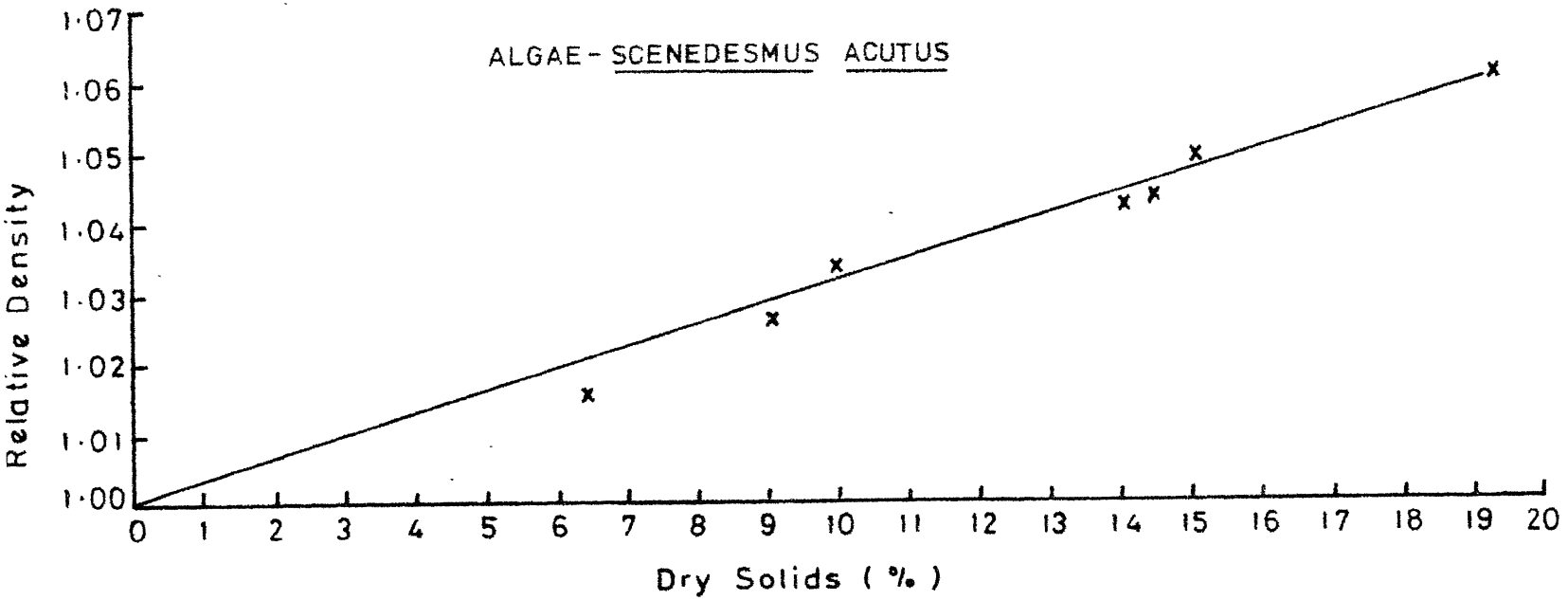


FIGURE 4·1·2 RELATIVE DENSITY VERSUS ALGAE (DRY) %

appropriate. Thick suspensions are encountered in centrifuges and driers.

a. METHOD

10 ml of algal suspension was taken in a conical bottom graduated centrifuge tubes, centrifuged at 5,000 RPM for 5 minutes, and the volume of wet algal cells determined. The wet packed cell volumes versus percent dry algae is plotted, and is shown in Figure 4.1.3. Dry % algae in suspension was determined by the method given in section 4.1.1 using centrifuge.

b. RESULTS AND DISCUSSIONS

Wet packed cell volume versus dry algae in suspension is plotted in Figure 4.1.3 The wet packed cell volume % was found about 10 times the dry algae % in suspension.

In packed cell volume determination experiments a brown layer was always found on the top of the algae sludge after centrifugation. This sludge was examined under microscope, and was found to be of empty algal cells without chlorophyll. Why these cells were coloured brown is not known.

4.1.4 VISCOSITY

The viscosity is one of the engineering parameters often used. Viscosity affects the flow of liquid and is used in solving flow, mass transfer and heat transfer problems.

a. EXPERIMENTAL METHOD

The viscosity of thin algal suspensions was determined

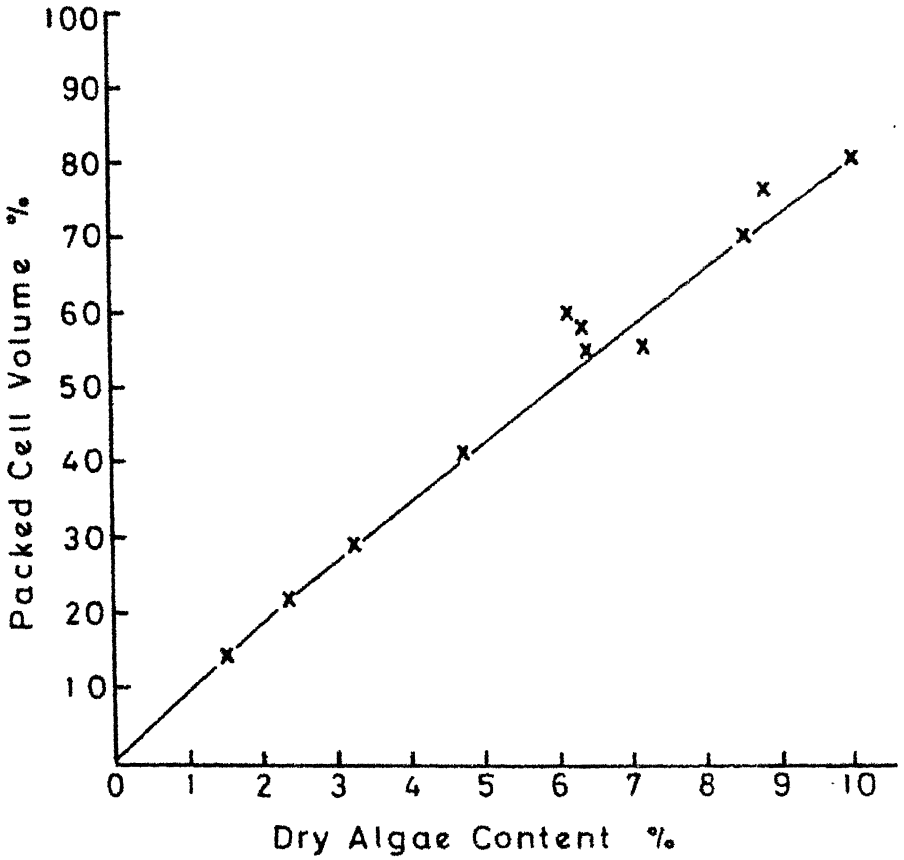


FIGURE 4·1·3 WET PACKED CELL VOLUME % VERSUS DRY ALGAE CONTENT %

by using Cannon Fenske Viscometer, and of thick slurry by co-axial cylinder viscometer (Rheostat - 2, Veg Kombinate Medizin - Und Labortectinik). The viscosities were determined at 27°C.

b. RESULTS AND DISCUSSION

The viscosity of thick suspensions is plotted against % dry weight in Figure 4.1.4. Centrifuged algae contain about 6% dry weight solids. Each point is the average of many readings, and the scatter around 1.5% is due to changing the cylinders in the viscometer.

4.1.5. LIGHT ABSORPTION COEFFICIENT

Light absorption coefficient for algal cultures is required to determine the optimum depth of cultures, and flow parameters. In this section the light absorption coefficient was determined. Standard Algal Assay Procedures do not specify the units in which radiations should be measured, although, the units reported are lux. Lux does not give much information. In this work lux-meter was used to measure the radiations, and approximate conversion factor from lux to watts/m^2 was obtained.

a. EXPERIMENTAL METHOD - The solar radiations available at different depths in algal cultures of different concentration were determined by using lux meter (O. Lang, Berlin). Light absorption coefficient was calculated by using Beer Lambert's law: $I = Io\ e^{-KCd}$, where, Io and I are light intensities at the surface of the culture and at a depth d in cms from the

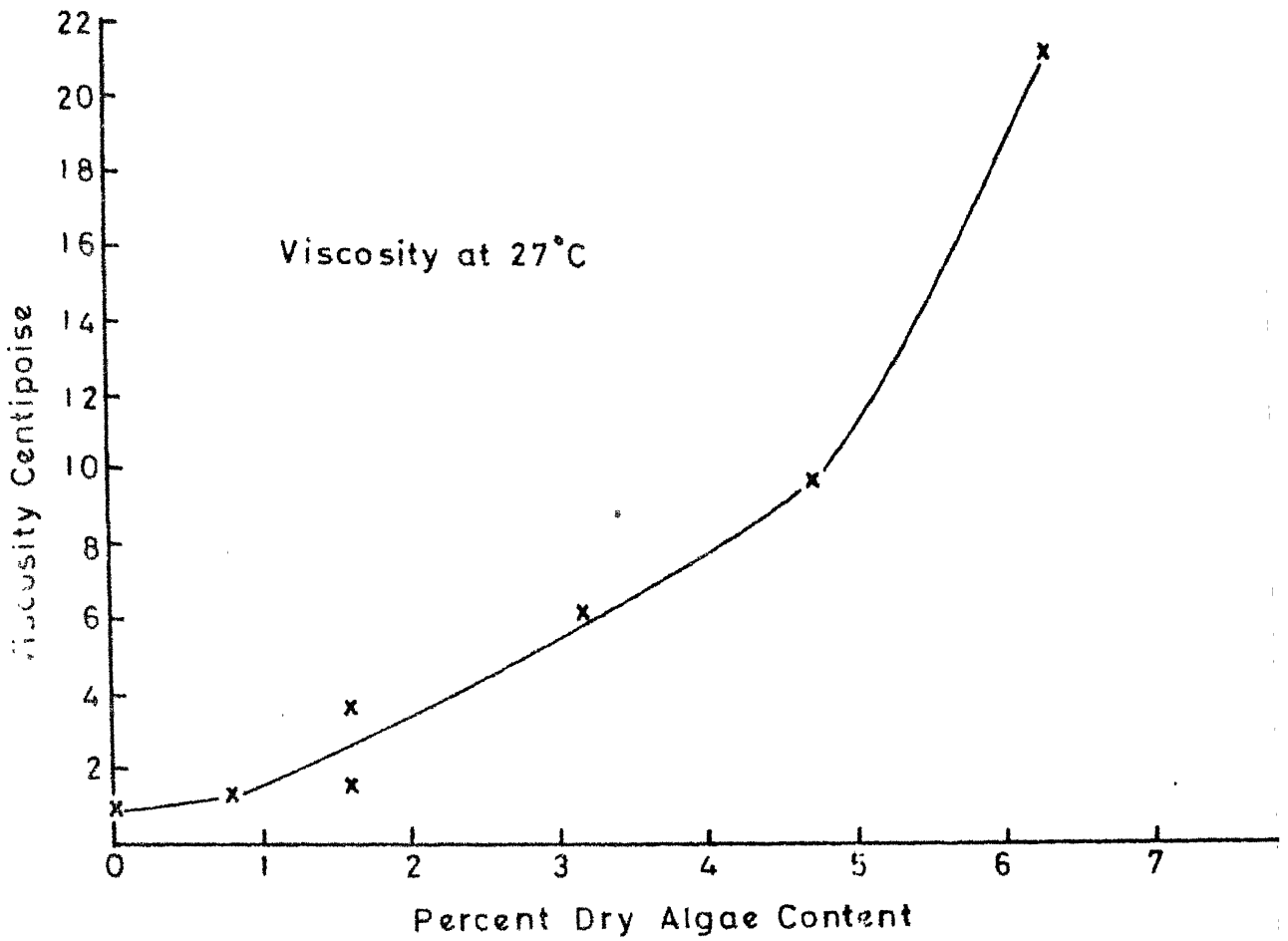


FIGURE-4·1·4 VISCOSITY OF THICK ALGAL SUSPENSION

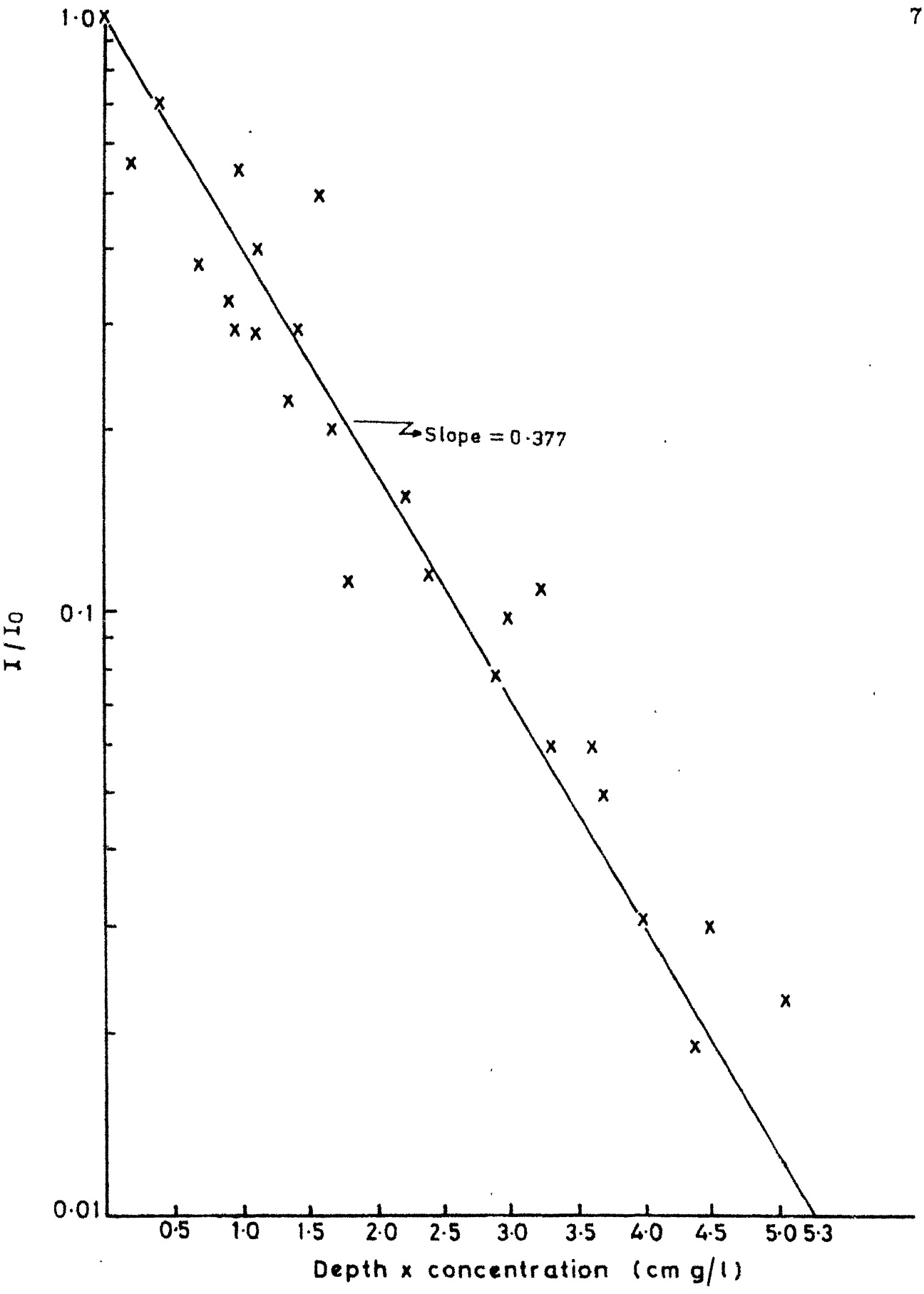


FIGURE 4·1·5 LIGHT ABSORPTION COEFFICIENT

surface and C is the concentration of algae in medium in g/l. For conversion factor determinations Joule meter (yellow spring Co., Ohio), and Lux meter (O Lang, Berlin) were used simultaneously.

b. RESULTS

The ratio of I/Io is plotted against the product of concentration in g/l and depth in cms on semilog paper, and is shown in Figure 4.1.5.

The absorption coefficient for Scenedesmus acutus was found to be 0.869 1/g.cm. Conversion factor under clear sky was 1 lux = .009 watt/meter^2.

Beer Lambert's law holds good for solutions. Algal cultures are not solutions but suspensions, and scattering of points in Figure 4.1.5 may be due to this only. However the analysis gives an average value of light absorption coefficient to use.

c. DISCUSSION

Tamiya et al., (1953) measured the value of extinction coefficient of Chlorella ellipsoida and found its value to be .41 1/ml cm. The value of coefficient calculated by them using the data of Emerson and Lewis was 0.23 1/ml cm. These values in terms of 1/g cm for Scenedesmus are 4.2 and 2.3 respectively. In the experiments of Tamiya et al., (1953) and Emerson, the value of extinction coefficient were determined at different wave lengths and average was taken. This method (Tamiya's

and Emerson's) of determination of extinction coefficient is sensitive for reflection, refraction, scattering of light, and collection efficiency of the equipment. The direct method of measuring light at different depths used in this work is free from these drawbacks and gives more realistic value of extinction coefficient to be used in field experiments. In algal cultures the saturation depth and compensation depths can be defined as the depth at which 85% and 99% of the radiations are absorbed. For this definition the saturation and compensation depths for algal culture of concentration 0.5 g/l are 8.8 cm and 10.6 cms respectively.

4.2. GROSS PARAMETERS

Effect of pH and nutrient concentration on growth, the gain in yield by mixing and aeration, the possibility of recycling the clear liquid from centrifuge to tanks for growth, and optimum inoculum size are the gross parameters considered. In this section the effect of gross parameters on the growth of algae is presented.

4.2.1. NUTRIENT CONCENTRATION

Algae require carbon, nitrogen, oxygen, phosphorus and trace elements for growth. Of these, nitrogen and phosphorus are considered as major elements governing the primary productivity of algae. Although considerable work has been done on the nutrition of microorganisms, adequate information concerning the inorganic requirement of algae seriously lags behind the progress made in the study of their organic metabolism (Hunter et al., 1950). The inorganic requirement of algae is reviewed by Krauss (1953), O'Kelley (1974), Kuhl (1974) and Stewart (1968). The role of nitrogen in eutrophication is reviewed by Horne (1971), and of phosphorus by Brown (1971) and Kuhl (1968).

Nutrient medium composition used in the cultivation of algae differs widely in total concentration and in relative amounts of individual element. In most of the cases it seems that nutrient media composition was chosen empirically. Table 4.2.1 shows the absolute and relative values of nitrogen and phosphorus used for algal cultivation by different workers.

TABLE 4.2.1

NITROGEN AND PHOSPHORUS CONCENTRATIONS AND THEIR RATIOS USED IN ALGAL CULTIVATION

Algae	Nitrogen source	P source	N g/l	P g/l	Ratio N/P	Reference
Chlorella	KNO_3 $Ca(NO_3)_2$	KH_2PO_4	0.206	0.056	3.64	Warburg Medium Vassnik et al., (1953)
C_p	KNO_3	KH_2PO_4	0.080	0.030	2.70	Benson medium Vassnik et al., (1953)
C_p	KNO_3	KH_2PO_4	0.168	0.280	0.60	Myer's medium Krauss (1953)
C_p	KNO_3	KH_2PO_4	1.39	0.278	5.0	Davis et al., (1953)
C_p	KNO_3	KH_2PO_4	0.476	0.19	2.48	Gummert et al., (1953)
C_p	KNO_3	KH_2PO_4	0.635	0.57	1.02	Gottesman (1953)
C_p	KNO_3	KH_2PO_4	0.346	0.57	0.60	Arthur et al., (1953)
C_p	KNO_3	KH_2PO_4	0.346	0.285	1.21	-do-
C_p	KNO_3	KH_2PO_4	0.35	0.558	0.627	Davis et al., (1953)
C_e	Urea	KH_2PO_4	1.68	0.285	5.9	Myers, et al., (1951)
C_e	Urea	KH_2PO_4	1.69	0.335	5.02	Mituya et al., (1953)
C_v	KNO_3	KH_2PO_4	0.35	0.56	0.625	Geoghegan, (1953)
Sq	Urea	KH_2PO_4	0.59	0.278	2.12	Davis et al., (1953)
Sq	Urea	KH_2PO_4	0.56	0.155	3.62	Ann Rep. 1968
Sa	Urea	Scenedin	0.074	0.022	3.52	IGAP
Mixed Cultures	Urea	KH_2PO_4	1.69	0.285	5.9	Swaminathan (1971)

Cp - Chlorella pyrenoidosa Ce - Chlorella ellipsoidea

Sq - Scenedesmus quadricauda Cv - Chlorella vulgaris

Sa - Scenedesmus acutus

In this section the effect of changing the urea and Scenedin concentration and their ratios on growth indoors and the effect of urea and Scenedin concentration, when they are in the ratio 1:2 outdoors are presented. The indoor experiments were conducted to find out the optimum nitrogen and phosphorus ratio and their absolute values for the growth of algae. Once having found this in indoor cultures it could be verified in outdoor cultures.

I. INDOOR CULTURES

a. EXPERIMENTAL METHODS

In culture tubes nutrient media of different composition was taken. The concentrations of urea and Scenedin taken and their N/P ratio are given in Table 4.2.2.

The tubes having nutrient medium of known composition were inoculated with the same amount of inoculum, kept in thermostat, illuminated continuously and aerated with air only. The growth and pH, were measured daily in the morning. Duplicate tubes were kept for each concentration.

b. RESULTS

The absorbance of the culture is plotted against time in Figure 4.2.1 A. Figure 4.2.1 B shows the pH of the tube culture at different time. The following are the important points to note:

1. Optical density in indoor cultures started decreasing after 5th day. pH of the medium also started decreasing,

TABLE 4.2.2

UREA AND SCENEDIN CONCENTRATION, AND N/P RATIOS USED IN INDOOR CULTURES

Sl. No.	Urea g/l	Scenedin g/l	Nitrogen mg/l	Phosphorus mg/l	N/P
1	0.1	0.2	74	22	3.4
2	0.2	0.2	130	22	5.9
3	0.3	0.2	186	22	8.4
4	0.1	0.4	92	44	2.1
5	0.2	0.4	148	44	3.36
6	0.3	0.4	206	44	4.68
7	0.1	0.6	110	65	1.7
8	0.2	0.6	166	65	2.8
9	0.3	0.6	222	65	3.4

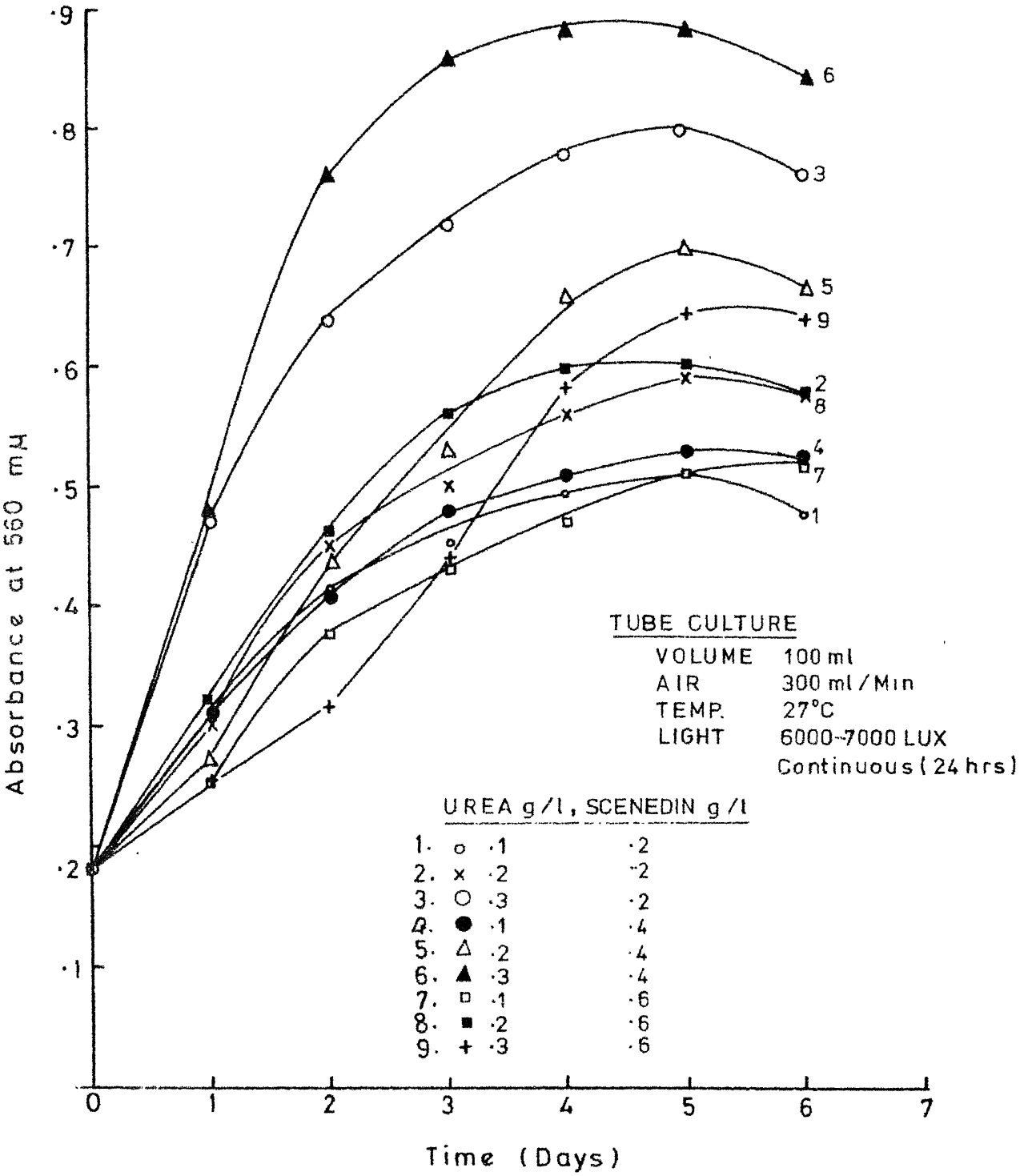


FIGURE. 4·2·1 A. EFFECT OF UREA AND SCENEDIN CONCENTRATION ON THE GROWTH OF ALGAE (INDOOR CULTURE)

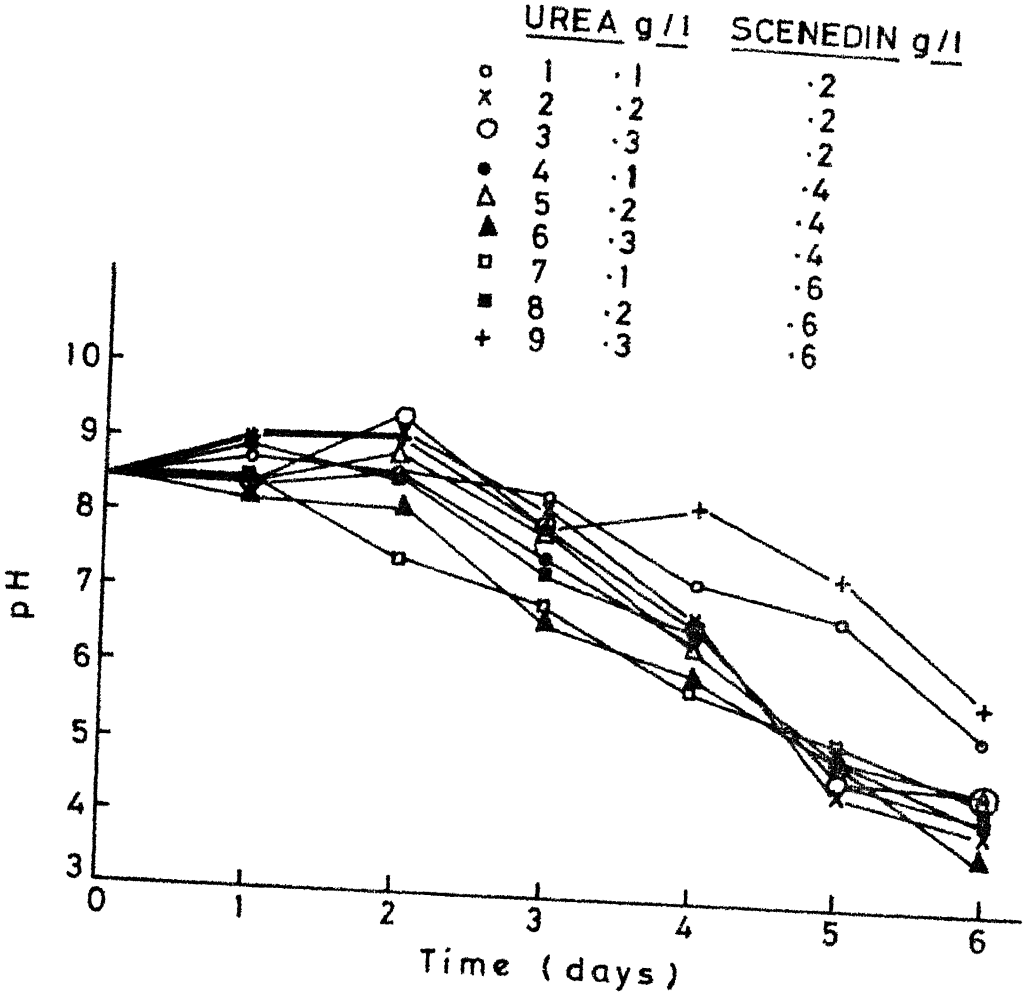


FIGURE 4·2·1·B pH OF THE TUBE CULTURE

and went down to 4 in few tubes. The decrease in pH and optical density was never found in outdoor cultures.

2. The algae were growing even after exhaustion of nitrogen. In all the cases growth increased with increase in initial nitrogen concentration of the medium.

3. Nutrient media containing 0.3 g/l urea and 0.4 g/l Scenedin gave maximum yield and growth rate.

c. DISCUSSION

The concentration of urea 0.3 g/l and Scenedin 0.4 g/l which gave maximum yield corresponds to nitrogen 206 mg/l and phosphorus 44 mg/l. Prible and Marva (1969) found optimum concentration of nitrogen between 100 to 300 mg/l for Scenedesmus quadricauda.

The lower growth in III set (0.6 g/l Scenedin) indicates that there are some elements in Scenedin which are toxic at higher concentrations. Scenedin 600 mg/l gives 27 mg/l sulphur and this concentration of sulphur may limit the uptake of phosphorus, which is 44 mg/liter. It is reported Prible and Marva (1969) that phosphate uptake is reduced if the concentration of sulphur is high.

II. OUTDOOR CULTURES

a. EXPERIMENTAL METHOD

Outdoor experiments were performed in C tanks. Carbon dioxide was fed at the rate of 500 ml/minute in each tank.

This was found in earlier experiments that this carbon dioxide rate is enough to make carbon dioxide non limiting. The experiments were performed in two series; in one series the initial concentration of urea was taken 0.1, 0.2 and 0.3 g/1, and of Scenedin, 0.2, 0.4 and 0.6 g/1 respectively, while in other the concentration of urea was 0.1 and 0.05 g/1, and of Scenedin 0.2 and 0.1 g/1. The optical density, pH and nitrogen were measured daily in the morning.

b. RESULTS

Absorbance of the culture against time is plotted and shown in Figure (4.2.1 C).

The important points are:

1. The growth in the medium containing 0.1, 0.2, 0.3 g/1 urea, and 0.2, 0.4, 0.6 g/1 Scenedin respectively is almost same.

2. The growth in medium containing 0.1 g/1 urea, 0.2 g/1 Scenedin, and 0.05 g/1 urea, 0.1 g/1 Scenedin is same. In another experiment it was found that protein content of algae vary from 63% to 30% depending upon nitrogen content of the medium, protein content are indicated in Figure (4.2.1 C).

3. Indoor and outdoor cultures are not comparable because of the different light pattern. Whether carbon dioxide was limiting in indoor cultures is not known.

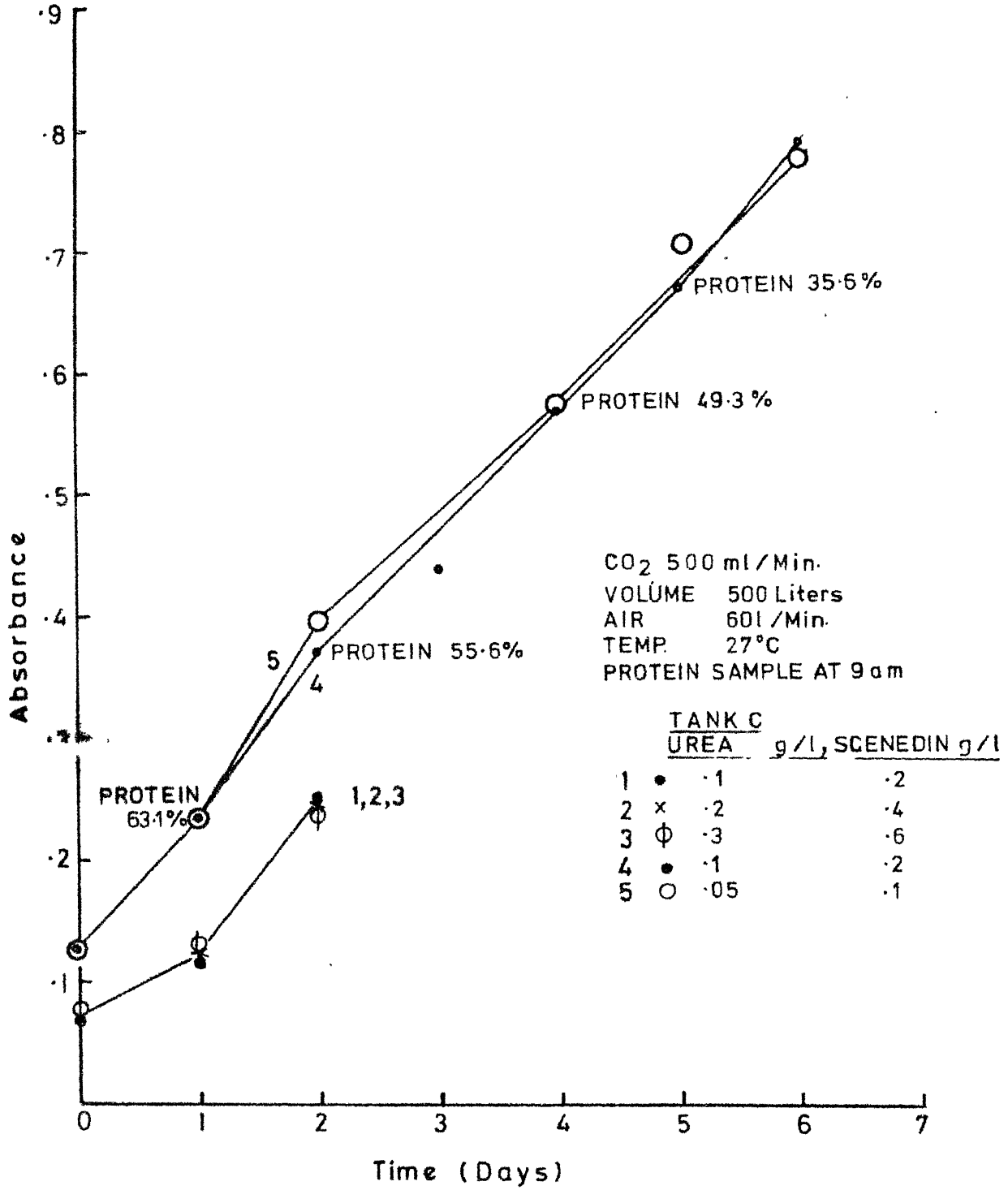


FIGURE. 4·2·1·C EFFECT OF UREA AND SCENEDIN CONCENTRATION ON THE GROWTH OF ALGAE (OUT DOOR CULTURE)

c. DISCUSSION

The results, that growth does not change with nitrogen concentration indicate that nitrogen is not limiting in outdoor cultures. This is possible because algae can readjust the nitrogen content of the cells. The change in protein content shown in Figure 4.2.1 C supports this hypothesis. Krauss (1953) has reported that protein content of algae can be varied from 8.7 to 58% by changing the amount of nitrogen supplied. The difference observed regarding the response to nitrogen concentration in indoor and outdoor cultures may be due to their different growth conditions. Outdoor cultures were grown under day and night (light 12 hr, dark 12 hr) cycle, while indoor cultures were grown under continuous illumination. Under light and dark cycle, outdoor cultures can readjust their nitrogen-carbon balance in night, and thus are not nitrogen limiting, but are photosynthesis limiting. The indoor cultures under continuous light are not able to readjust the internal nitrogen-carbon balance and are thus limited by nitrogen supply. However, it must be noted that the two cultures can be compared on either total light received or on the basis of light-dark programming. The conclusion here is arrived at on the former basis.

d. CONCLUSION

The indoor and outdoor cultures are not strictly comparable because of the fact that light conditions were

different, and it is not known that whether carbon dioxide was limiting in indoor cultures. Furthermore indoor cultures seem to be inhibited by sulphur. In outdoor experiments it was found that:

1. Bio-mass yield and rate did not depend upon nitrogen level.
2. A high amount of Scenedin was not inhibiting.
3. Even though biomass yield was not affected (at different level of supplied nitrogen) protein value of the algae changed. Thus it seems that one can feed nitrogen and phosphorus in fairly high absolute and relative concentration in order to keep the protein value and growth rate high. A logical extension of this experiment would be to feed nitrogen and phosphorus intermittently, instead of all at one time so that there will be optimum utilization of these essential factors.

4.2.2. HYDROGEN ION CONCENTRATION AND BICARBONATE UTILIZATION

Hydrogen ion concentration in algal cultures is important because it affects:

1. Metabolic functions of the cell
2. CO_2, HCO_3^-, CO_3^{--} distribution, and
3. Solubility of nutrients.

In outdoor cultures it is not economical to grow algae in pH buffer media at optimum pH. The possible alternatives

are: 1) to grow algae at optimum pH (if there is any) adjusted by adding acid, and 2) growing algae without pH adjustment.

In this chapter the possibility of using either of the two above is tested. Increase in carbon dioxide feed rate lowers the pH and increases the growth rate. The increase in growth rate may be either due to increased carbon dioxide concentration in the culture, or due to decrease in pH which makes the nutrient salts (phosphorus or iron) more soluble, and thus available for algal growth. At particular rate of carbon dioxide feed the effect of lowering the pH of the medium by adding acid is examined in this section.

The reports in the literature on the utilization of bicarbonate by algae are conflicting (Rabinowitch 1945). Österlind (1948, 1950) has demonstrated that Scenedesmus can utilize bicarbonates. Gianelli (1971) tried to use $NaHCO_3$ as carbon source in miniponds treating nitrate waste by growing Scenedesmus quadricauda, and found that Scenedesmus cannot use bicarbonate. Important point to note is that in Gianelli's system the concentration of nutrients was very low.

The pH of the tap water and culture media in our experiments was 8.3. Most of the carbon at pH 8.3 is in the form of bicarbonate only, and Scenedesmus grow at this pH. The question then is, can Scenedesmus acutus utilize

bicarbonate as a source of carbon. If Scenedesmus acutus can utilize bicarbonate ions, then the cultivation of Scenedesmus at higher pH would reduce the desorption of carbon dioxide from culture to atmosphere. The possibility of using bicarbonate carbon of sodium bicarbonate as a source of carbon in Scenedesmus acutus cultivation is presented in this section.

a. EXPERIMENTAL METHODS

All the experiments were done in 6 tanks with 500 liter nutrient medium containing 0.2 g/l urea, and 0.4 g/l Scenedin. The tanks were started with same initial inoculum concentration, and were without external supply of carbon dioxide. In one tank pH was adjusted between 7.5 and 8.0 by adding commercial hydrochloric acid. In other tank pH was not adjusted. Absorbance of the culture and pH were measured daily.

In another set of experiments tanks were prepared in similar fashion, and equal amount of carbon dioxide (300 ml per minute from 9 A.M. to 5 P.M.) was put into each tank. In one tank pH was adjusted to lower value (6 to 6.5) by adding HCl, while in other pH was not adjusted. In the same series a tank was run with $NaHCO_3$ as carbon source (no carbon dioxide). In this tank with $NaHCO_3$ pH was adjusted by adding HCl.

b. RESULTS -

Absorbance versus time for the above experiments are plotted in Figure 4.2.2 A and 4.2.2 B.

Some important points to note are:

a. There is no change in growth of algae by lowering down the pH of the medium by adding acid, when extra carbon dioxide was not added.

b. At pH higher than 9.5 algae starts forming clumps, but growth rate was not affected.

c. There is no effect of lowering down the pH by adding acid on the growth of algae in carbon dioxide fed cultures.

d. Scenedesmus acutus can use bicarbonate carbon as a carbon source (see Figure 4.2.2 B).

e. The growth rate of algae with 200 grams $NaHCO_3$ per day is same as in the tank with 300 ml/minute carbon dioxide, 8 hr/day.

c. DISCUSSION

I. Effect of pH on growth (without external carbon dioxide)

The addition of acid to decrease the pH disturbs the carbon dioxide balance, and makes the carbon of HCO_3^- and CO_3^{--} available in the form of CO_2 for the growth of algae. The solubility of carbon dioxide at higher pH is higher, but concentration of free carbon dioxide is low because most of the carbon is in the form of HCO_3^- and CO_3^{--}. At higher pH the transfer of carbon dioxide from the atmosphere

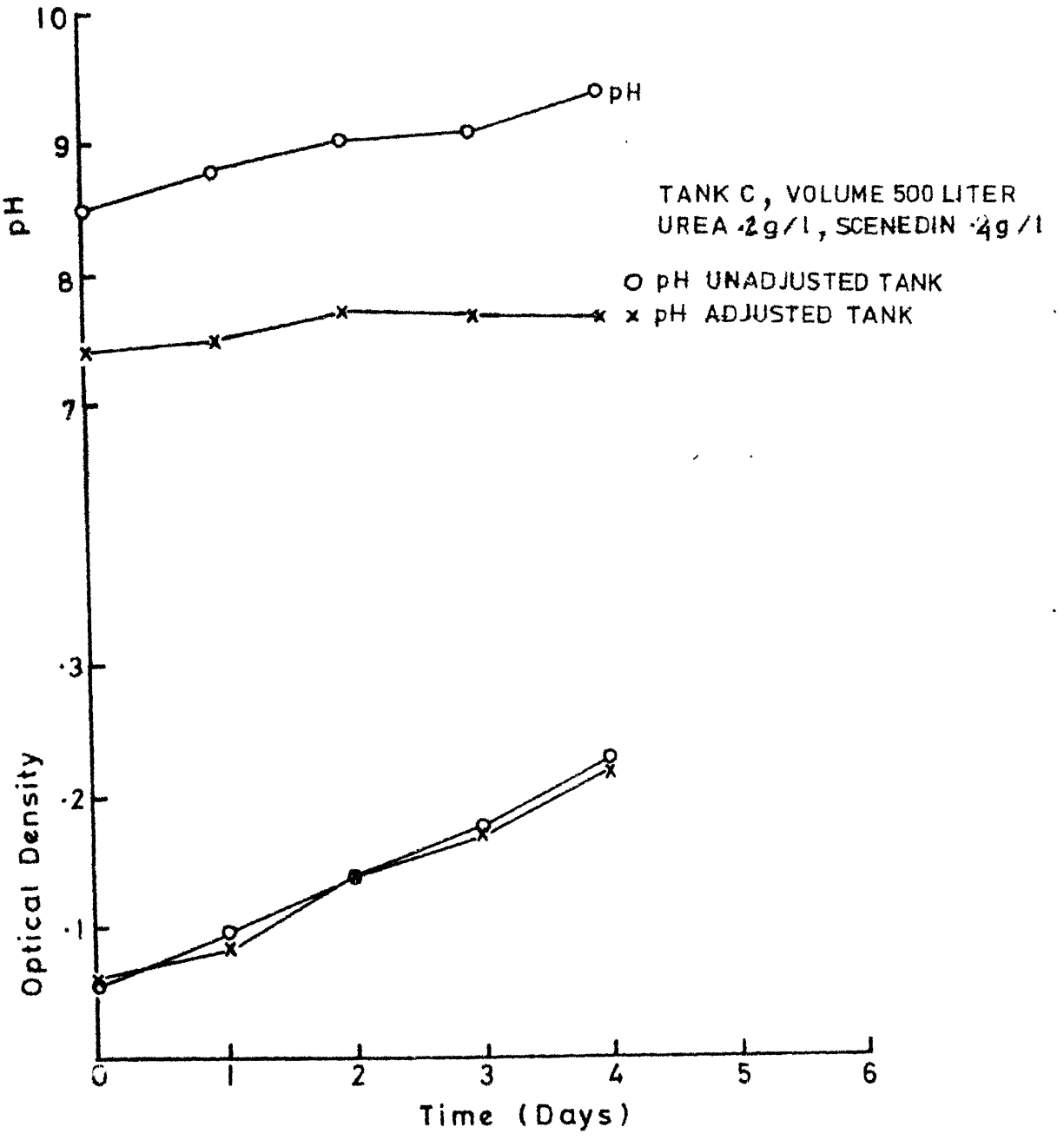
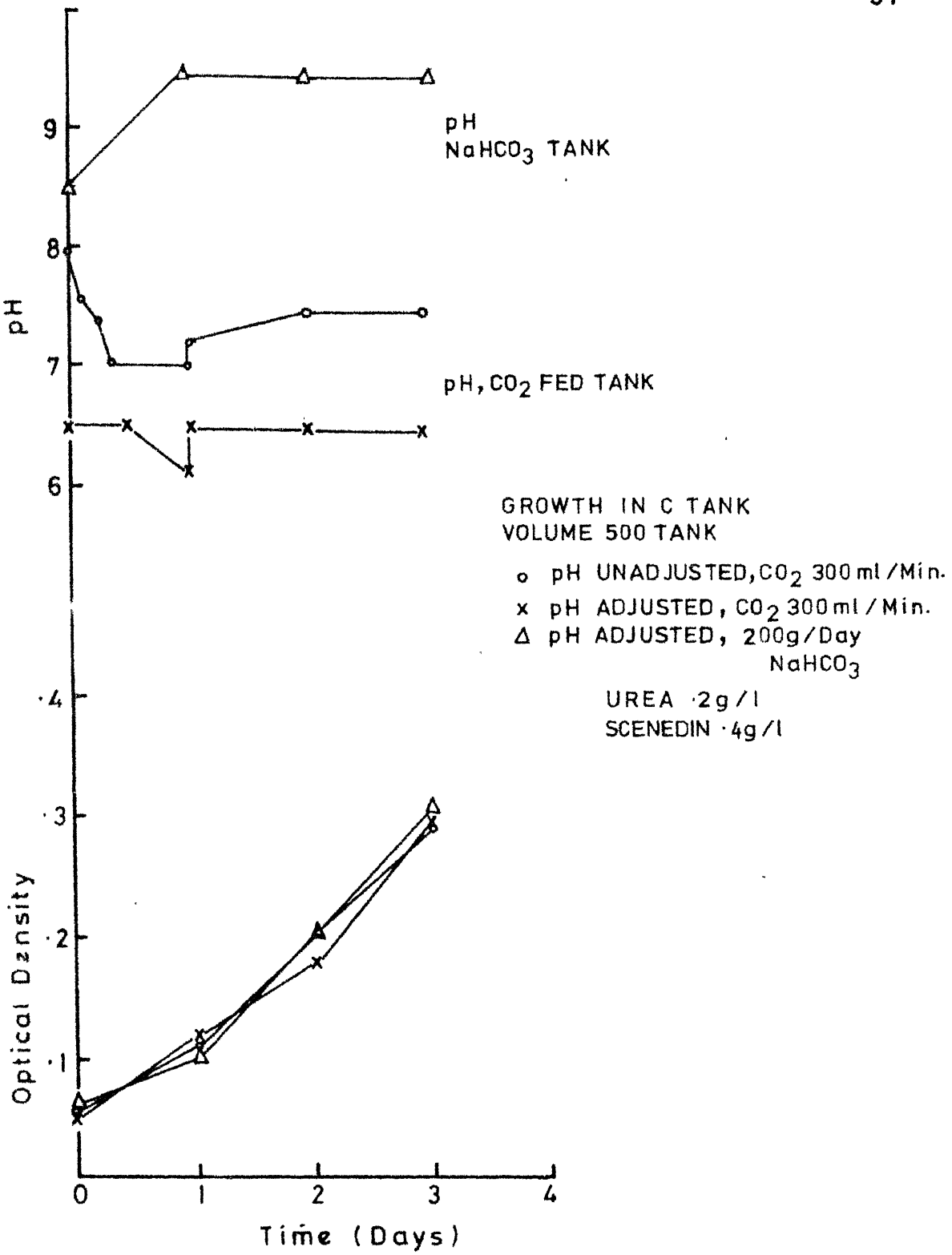


FIGURE · 4·2·2·A EFFECT OF pH ON THE GROWTH OF ALGAE



FIGURE·4·2·2·B EFFECT OF pH ON THE GROWTH OF ALGAE IN CO_2 FED TANKS

to culture is higher because of the chemical reactions to give HCO_3^- etc. The increased supply of carbon in cultures where acid is not added may compensate for decrease in the growth of algae, if there is any due to metabolic inhibition at higher pH. It seems that in cultures without external supply of carbon dioxide, cultures are carbon dioxide limiting.

At higher pH the nutrient salts (phosphorus and iron) may be removed by precipitation, affecting the growth of algae. At pH higher than 8.4, only dibasic orthophosphate (HPO_4^{--}) is available. It seems that algae Scendesmus acutus use preferentially this form of phosphorus which is probably available from Scenedin.

II. Effect of pH on carbon dioxide fed cultures

Growth rates of Scenedesmus acutus increase (although efficiency of carbon utilization decreases) with increase in carbon dioxide feed rate (this is discussed in Chapter 8). Higher carbon dioxide feed rates increase the concentration of carbon dioxide, and lower the pH of the culture. The increase in growth rate of algae at higher carbon dioxide feed rate may be either due to higher carbon dioxide concentration or due to lower pH, which may make the nutrients more soluble. The results of this experiment show that increase in growth rate of algae is probably due to higher concentration of carbon dioxide in the culture, and not due to the lower pH.

III. Utilization of Bicarbonate

Bicarbonate provides a buffer to the pH rise and can supply utilizable source of carbon to algae. We found in our experiments that Scenedesmus acutus does use bicarbonate (Figure 4.2.2 b). It seems that either bicarbonate or undissociated ion can pass through cell membrane.

The growth rates of algae in tanks getting 200 g/day sodium bicarbonate, and 300 ml/minute carbon dioxide for 8 hours a day were same. 200 grams $NaHCO_3$ corresponds to 28.5 grams carbon, and 300 ml/minute carbon dioxide for 8 hours a day is equal to 70.1 grams carbon per day. The carbon fixed in algae (calculated on the basis of the growth in 3 days and 50% carbon in algae) was 20.8 grams carbon/day. The efficiencies of $NaHCO_3$ carbon utilization by Scenedesmus acutus were found 72% when calculated on the basis of carbon fixed in algae in $NaHCO_3$ fed cultures, and 36% when calculated on the basis of carbon fixed in $NaHCO_3$ fed culture minus carbon fixed in algae in control experiment. In cultures with external supply of carbon dioxide the pH of the cultures were low, and there was desorption of carbon dioxide from the culture leading to the lower efficiency of carbon dioxide - carbon utilization. The efficiencies of carbon dioxide-carbon utilization were calculated in the same way as those of $NaHCO_3$ and were found to be 30% and 15%.

Litchfield and Hood (1964) have demonstrated the presence of carbonic anhydrase in algae. This enzyme can catalyze the

dehydration of HCO_3. The addition of carbonic anhydrase which can be derived from algae grown in carbon dioxide limited cultures may further improve the utilization of bicarbonate and growth of algae in cultures without external supply of carbon dioxide.

d. CONCLUSION

The conclusions derived from these experiments are:

1. In outdoor cultures without external carbon dioxide supply, the growth of algae is limited by carbon dioxide supply from air, and is not limited by metabolic inhibition upto pH 9.5.

2. Lowering down the pH in cultures with external carbon dioxide supply does not affect the growth.

3. Algae Scenedesmus acutus does utilize sodium bicarbonate as a carbon source. The effect of adding small amount of carbonic anhydrase in algal cultures needs to be developed. Algae grown in the absence of carbon dioxide may serve as a source of the above enzyme.

4.2.3. MIXING AND AERATION

Mixing in tanks affects the growth of algae in one or more of the following ways:

1. Metabolic stimulation
2. Mass transfer
3. Light dark pattern

The stimulation of metabolic rates of algae by mixing is known from the time of Rutner, 1926,(Doty, 1971). The effect of turbulence on metabolic rates is discussed by Soeder and Stengel (1974). The effect of mixing on mass transfer rates is reviewed by Thackston (1969). The random turbulence caused by mixing can change light-dark pattern of algal cultures. Kok (1953) and Fredrickson (1969) have discussed the effect of light-dark pattern and turbulence on the growth and photosynthesis of algae. Theoretically the improvement in efficiency is expected, but some of the experimental results reported in the literature do not support this. Wassnik (1953) did not find any beneficial effect of mixing in light in summer. Miller et al., (1970) found in outdoor experimental channel using river water that the growth of biomass measured as chlorophyll 'A' does not increase with the increase in velocity from 0.25 ft/sec to 1.40 ft/sec. The growth of Anabaena cylindrica and Anabaena spiroides is inhibited by turbulence (Fogg and Tan-Tun 1960; and Volk and Phinney 1968).

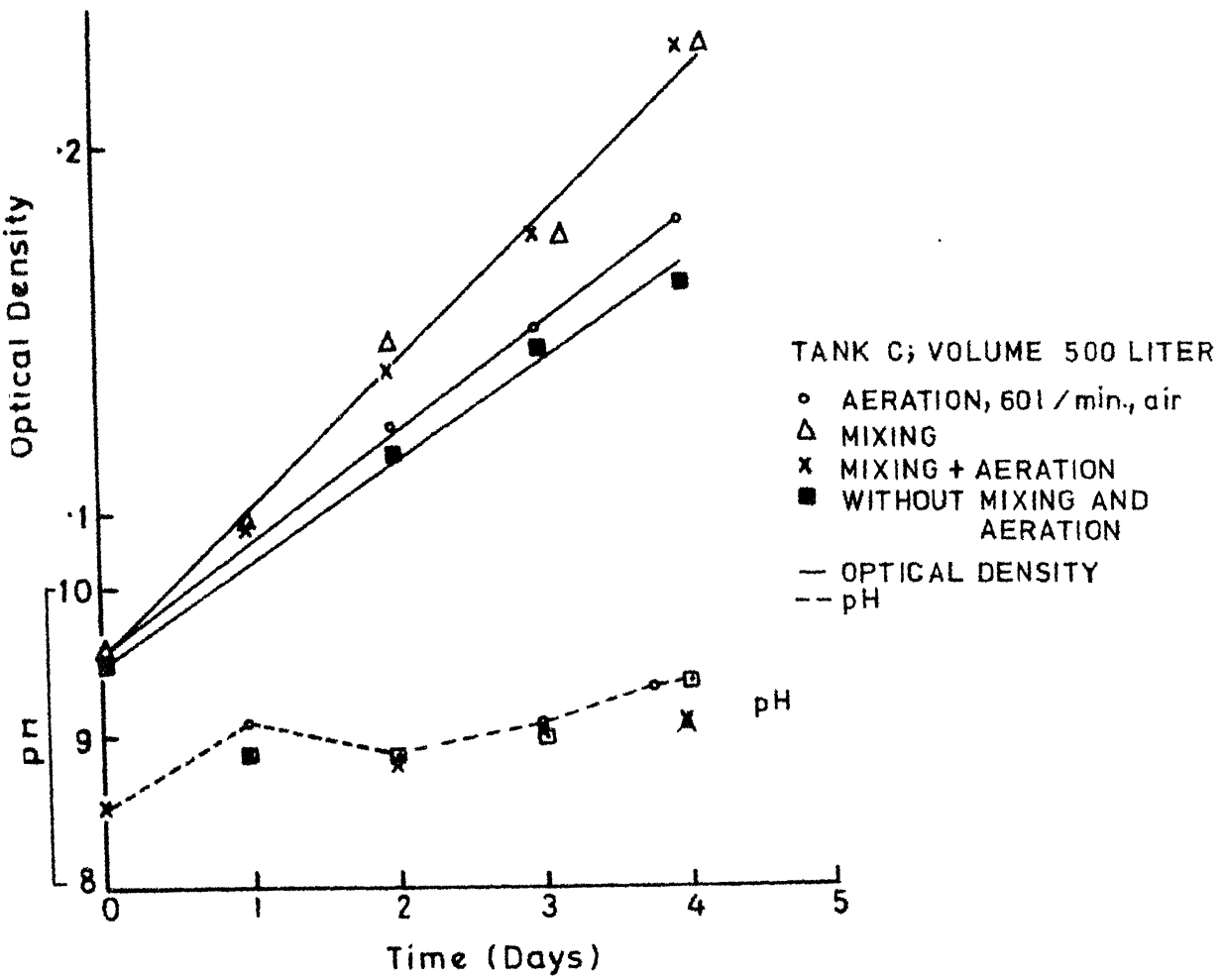


FIGURE 4·2·3 EFFECT OF AERATION&MIXING ON THE GROWTH OF ALGAE

TABLE 4.2.3.1

GROWTH IN TANKS WITHOUT AERATION AND MIXING, WITH AERATION; MIXING, AND MIXING + AERATION

Tank	Growth rate g/m^2 day	% increase	Evaporation l/m^2 day	% Evaporation % of total volume
Without mixing and aeration	2.1	-	6.92	1.34 %
Aeration	2.42	15.4	7.68	1.53
Mixing	3.23	53.6	10.68	2.13
Mixing and Aeration	3.23	53.6	10.8	2.13

TABLE 4.2.3.2

DISSOLVED OXYGEN IN TANK CULTURES AT NIGHT

Tank	Treatment	Algae concentration g/l	Dissolved oxygen concentration mg/l
	Tap water	0	5.8
	Water saturated with air	0	7.86
C	With Mixing	0.13	7.5
C	Without mixing	0.13	0.15
B	With mixing	0.12	7.7

At such low growth rate of algae (without external carbon dioxide) there was growth of mites in the algal cultures. In tanks with mixing it was very easy to remove larvae of mites by putting a wire mesh across the flow, and removing the mite eggs by taking out the screen and washing it. In the tanks without mixing it was very difficult to remove mite cells. The mite larvae form a sheath of algae around them and grow in it. The contamination by mite was more in summer, and almost no contamination in winter. Probably the growth of mite was retarded by low night temperature in winter or larvae were killed by 10 degree difference in night and day temperature in winter. Increasing the temperature of the culture in summer for 1/2 to 1 hour so that it is injurious to larvae but not to the algae may be practical solution to reduce the growth of mites. Algae in unmixed tanks after 4 days were settled.

d. CONCLUSIONS

1. There is no gain by adding aeration over mixing in algal cultures.

2. Aeration by paddle wheels is enough to keep the algal cultures aerobic in night.

3. Mixing is necessary to keep the concentration of algae uniform, and to remove mite larvae by using wire screen across the flow.

4.2.4. RECYCLE OF CLEAR LIQUID FROM CENTRIFUGE

For economical commercial production of algae, the question of conserving water is very important. In this set of experiments the possibility of using clear liquid from centrifuge after harvesting the algae for algal culture was tested.

a. METHOD

I. Indoor Experiments - The culture tubes containing fresh medium and medium prepared in centrifuged clear liquid were inoculated by equal volumes of algal culture. The concentration of urea and Scenedin in fresh and centrifuged medium was 0.1 g/l and 0.2 g/l respectively. The tubes were aerated and illuminated continuously. The optical density and pH were measured daily after making up the volume of culture medium. Triplicate tubes were kept for each set of tubes.

II. Outdoor Cultures - Outdoor experiments were carried out in A, B and C tanks. The growth rates of algae in nutrient medium prepared in tap water, and nutrient medium prepared in centrifuged clear liquid were compared. In one set of experiment the growth in fresh medium, and in centrifuged liquid after 2 cycles of growth was compared.

b. RESULTS

Optical densities of indoor culture and outdoor culture against time are plotted in Figure 4.2.4. One set of data out

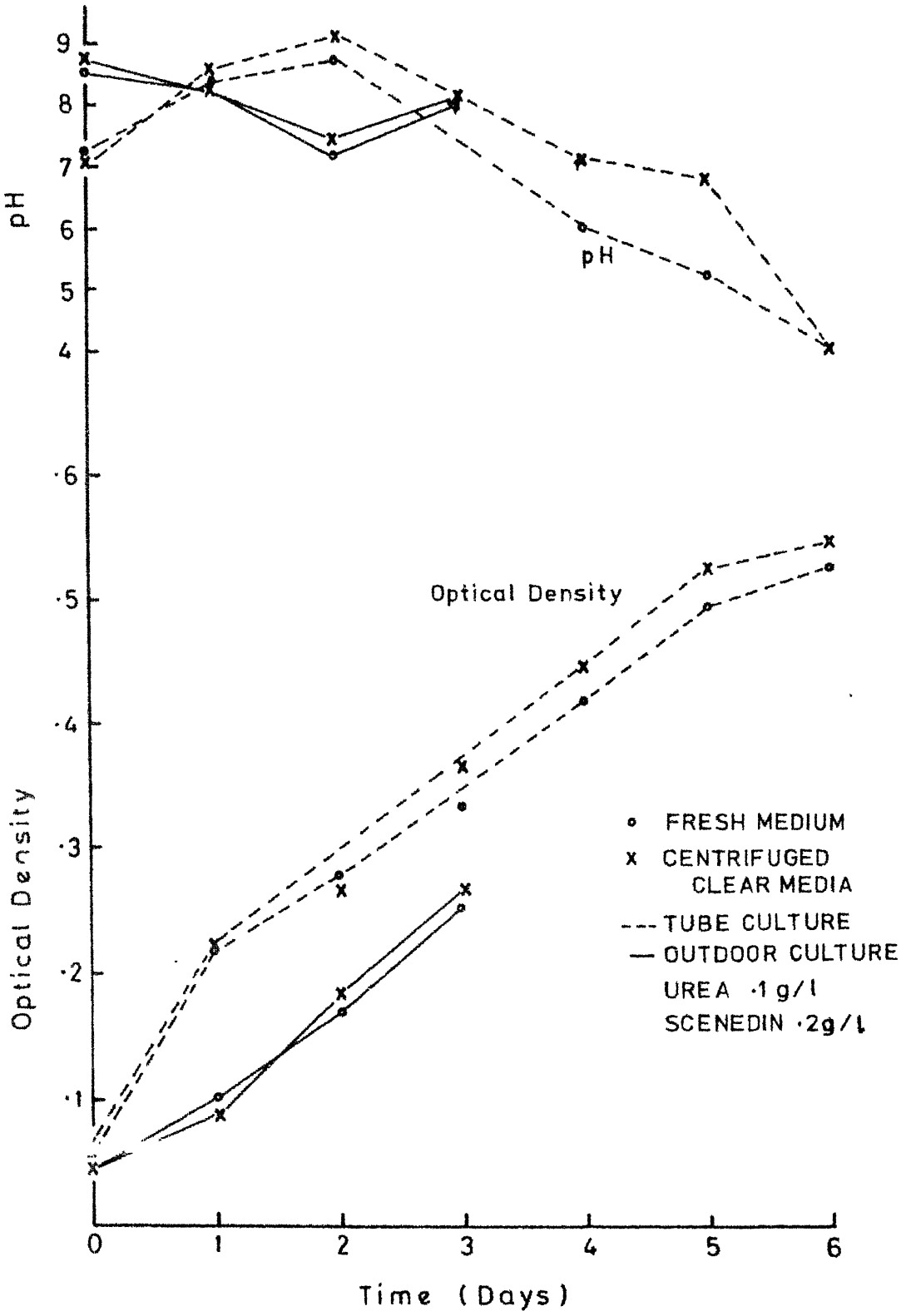


FIGURE-4·2·4 GROWTH IN CENTRIFUGED CLEAR LIQUID MEDIA

of three is plotted. There was no difference in growth of algae grown in centrifuged clear liquid, and fresh medium in each set.

c. DISCUSSION - The growth of algae in fresh medium, and in centrifuged clear liquid medium was found the same in indoor cultures. However, the tubes containing centrifuged clear liquid medium were found heavily contaminated by bacteria at the end of the experiment. In some tubes containing centrifuged clear liquid media the growth was found better than in freshly prepared nutrient medium, and this may be either due to carbon dioxide produced by bacterial contaminants from organic materials, or due to extra cellular materials (growth promoting) produced by algae during growth.

The outdoor tank cultures also did not show any difference in growth rate of algae in fresh and centrifuged clear liquid medium. There were some empty algal cells in the centrifuged clear liquid recycled to tank after growing algal culture twice, but there was no difference in growth rate when compared with growth in freshly prepared medium.

The results of this experiment indicate that algae do not produce any autotoxin, and water from centrifuge can be reused for algal cultivation. In water recycled algal systems, higher concentration of nutrients can be maintained even upto harvesting time to have desired quality of algae.

d. CONCLUSION

The conclusion derived from this section are:

1. Algae do not produce any autotoxin.

2. The clear liquid from centrifuge can be recycled to algal tanks.

4.2.5. INOCULUM SIZE

The optimum initial concentration of algal cultures to start with is important for economical cultivation of algae. Very low initial concentration of algae to start algal cultures may be lost due to photooxidation. Very high initial concentrations of algae result in high losses due to respiration and inefficient use of light energy due to shading.

In this section investigations on optimum initial concentration of algae culture to start with are presented.

a. METHOD

The experiments presented in this section were done in B and C tanks. The cultures with different initial algal concentrations were started with higher concentration of urea and Scenedin (0.2 g/l and 0.4 g/l) to make sure that nutrients are not limiting. Optical density and pH were measured daily. Experiments were done in duplicate and average is plotted. Optical density divided by initial optical density is plotted in Figure 4.2.5 B.

b. RESULTS AND DISCUSSION

The gain in algal biomass (OD at nth day - OD at (n - 1)th day) is plotted against initial absorbance and is shown in Figure 4.2.5 A. The optimum initial concentration of algae to start with was found to be between 200 mg/l to 300 mg/l. The specific growth rate of algae decreases with increase in initial optical density (Figure 4.2.5 B) but the gain in biomass shows an optimum initial concentration of algae to use. This initial optimum concentration of algae may be a function of liquid culture, depth, and carbon dioxide fed to the cultures. This optimum was determined for 13.4 cm culture depth, and 500 ml/minute carbon dioxide (8 hr/day) in B tanks. 200 mg/l initial concentration was found optimum for C tank also (depth 12.45 cms, and carbon dioxide 200 ml/minute, 8 hrs a day).

d. CONCLUSION

The specific growth rate of algae decreases with increase in initial concentration of algae, but the gain in algae mass does show an optimum at inoculum concentration of 200 mg/l in B and C tanks. Other things being equal, the experiment shows that there is an optimum inoculum concentration. However, to minimize the cost of production per unit biomass all the costs such as the cost of laboratory inoculum, the cost of harvesting and the cost of production in outdoor tank must be taken into account.

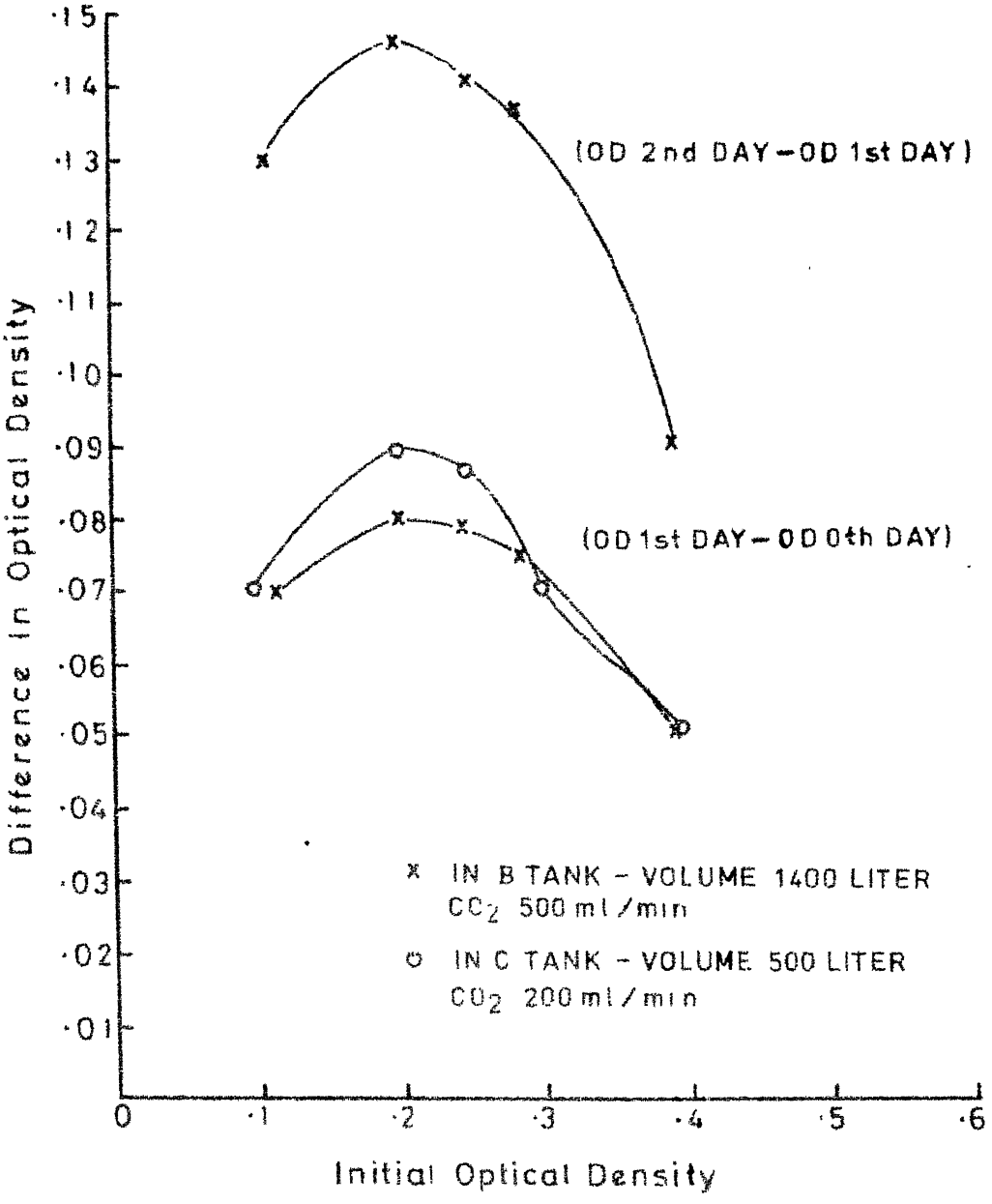


FIGURE-4·2·5·A EFFECT OF INOCULUM (INITIAL CONCENTRATION OF ALGAE) ON GROWTH

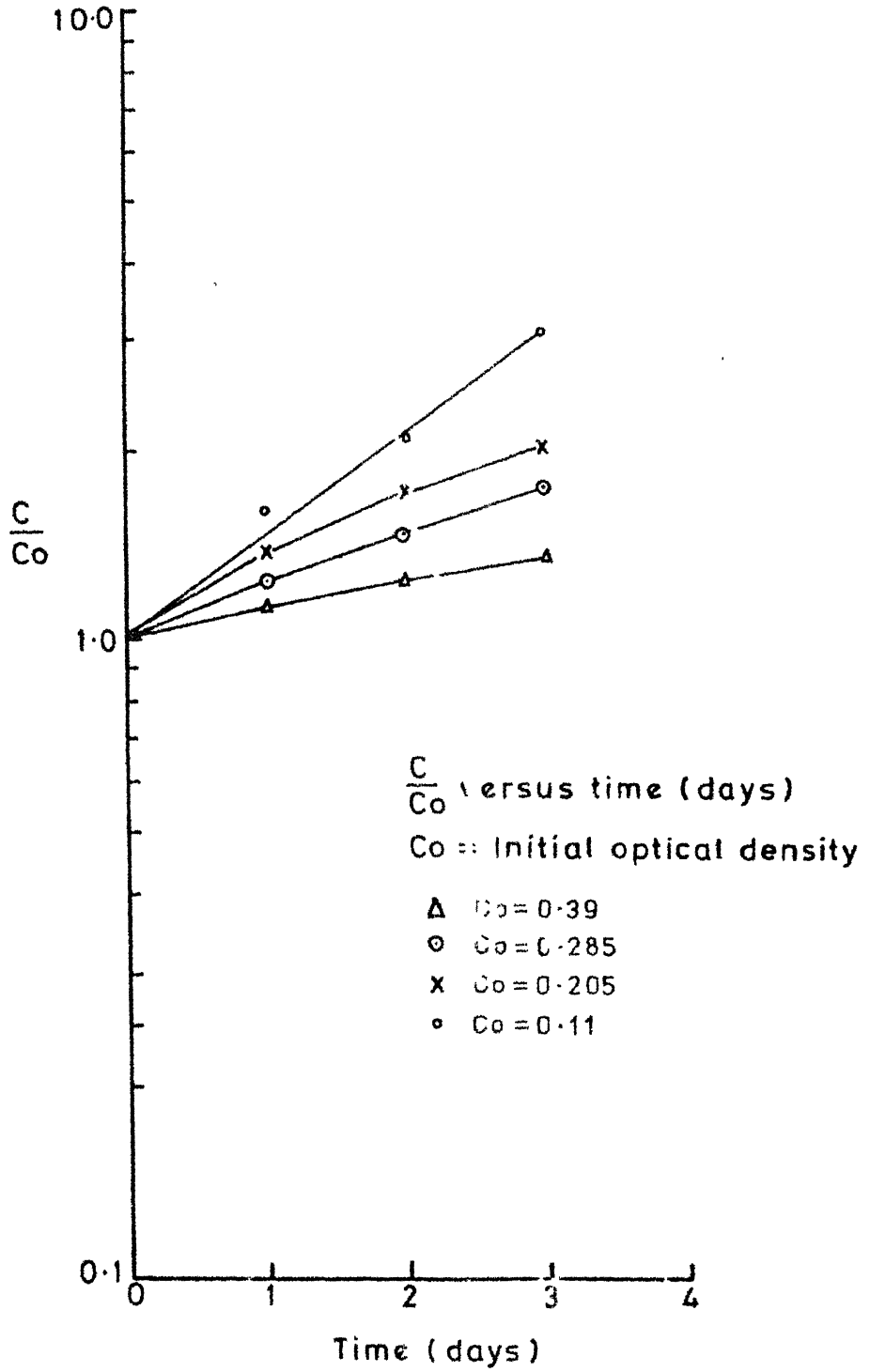


FIGURE 4·2·5B EFFECT OF INOCULUM SIZE ON THE SPECIFIC GROWTH RATE OF ALGAE

4.2.6. HARVESTING

As discussed earlier, the harvesting of thin algal cultures is still an unsolved problem. The algal cultures are harvested at IGAP by using centrifuge (Westfalia seperator SA 7-06 076), and then drying the thick slurry from centrifuge on an electrically heated drum drier (Escherwyss Model ESI. SS). The advantages of using a drum drier for drying algae to reduce the moisture to a level where it can be stored safely for a longer time are: 1. Dryingtime is less, and thus the losses of vitamins, and amino acids during processing are low. (Appendix 1 B shows the B.V., N.P.U., and D.C. of drum dried, sun dried, and cooked and sun dried algae). 2. The sudden change in temperature breaks the cell wall of algae making the protein etc., in the cell available for human system, and 3. Sterilizing the algae.

4.2.6.1. CENTRIFUGE

Centrifuge used was operated at different flow rates, and it was found that centrifuge gives 100 fold concentration giving slurry containing 3 to 5% dry solids. Algal slurry containing this much solid was easy to spray on drum drier.

4.2.6.2. DRUM DRIER

The heat transfer coefficient for the drum drier was calculated on the basis of two experiments. Heat transfer coefficient calculations are given in Appendix (6) and its value was:

Heat transfer coefficient = 12 cal/cm^2 hr °C

This drier at optimum conditions would dry approximately 20 liter algal slurry per hour per $meter^2$ of the drum drier.

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SUMMARY AND CONCLUSIONS FROM EXPERIMENTS OF PART I

The following properties were studied here:

1) Optical density of cultures versus dry weight of solids.
2) Densities of cultures, both thin and thick slurries.
3) Viscosities of thin and thick slurries.
4) Light absorption studies in situ in outdoor cultures.

The effect of the following gross parameters were studied:

1) Nutrient concentration, and N/P ratio.
2) Hydrogen ion concentration and bicarbonate utilisation.
3) Mixing and aeration.
4) Recycle of centrifuged clear liquid.
5) Inoculum size in outdoor cultures.

In addition, drum drier heat transfer calculations were carried out. All the above studies were necessary to establish the importance of all the engineering parameters for scale-up studies.

The major conclusions from Part I are as follows:

1. The density of algal slurries is not much different from water for practical purposes other than centrifugation. Since the operation of continuous centrifuges depends on slight differences in density between thick algal slurries

and water, a relationship has been obtained between the relative density of such slurries and water.

2. The viscosity of slurries of algal cultures has been determined for slurries upto 6% (by weight) solids. This viscosity is important for calculation of mass and heat transfer to thick slurries.

3. The depth of algal cultures for optimum light absorption in situ has been obtained. The light absorption coefficient was 0.87 1/g cm. Saturation and compensation depths for algal culture of 0.5 g/l algae were 3.8 cm and 10.6 cm respectively.

4. The quantity of nitrogen supplied does not seem to affect growth in outdoor cultures. The rate and yield remain the same over a 6-fold variation of nitrogen supplied. However, the protein-carbohydrate ratio seems to adjust itself to a nitrogen-limiting situation such that total biomass yield remains the same.

5. The usual practice of adding acid to decrease pH of algal cultures does not seem to affect the growth rate, whether external carbon dioxide is added or not. However, above pH 9.5 the precipitation of nutrients e.g., phosphate may affect the growth rate. Correspondingly algal cultures of Scenedesmus acutus seems to be able to utilize efficiently dissolved bicarbonate provided the pH is not above 9.5.

6. When external carbon dioxide is not added the effect

of aeration over mixing with paddles is negligible. Hence the use of air to provide extra oxygen or carbon dioxide is not necessary. When external carbon dioxide is added in the form of pure carbon dioxide then aeration obviously will contribute no carbon dioxide because of the low concentration of carbon dioxide in air.

7. Clear centrifuged liquid can be reused upto two times to grow algae, hence saving valuable water and salts for the cultures. Undoubtedly in large scale cultures some amount of fresh water should be added and same amount of centrifuged media should be purged, to cut down on the concentration of toxic substances, if any.

8. Other things being equal, there is a higher yield by taking an optimum inoculum concentration of 200 mg/l, in Scenedesmus acutus. The time-cost-optimum inoculum concentration will have to take care of all factors including harvesting and drying costs. The reported value may not be the same for this case.

PART II

FINE PARAMETERS

CHAPTER 5

5.1. MIXOTROPHIC GROWTH

Many species of algae can grow in dark using organic carbon for their energy and cell synthesis. Droop,(1972) has given a list of heterotropic and autotrophic algae. Algae of the genera Chlorella and Scenedesmus can grow photosynthetically, but many strains can also grow in the dark utilizing organic carbon (Denforth 1962). It was thought that the growth of algae can be increased by making it to grow in dark on organic reduced carbon and in light by photosynthesis. Such growth has been named "mixotrophic" growth.

In the experiments presented in this chapter, organic carbon (molasses, or glucose) was added to the algal cultures in evening in the hope that some growth could be promoted even at night. This would then enable us to have a 24-hour growth period, instead of a 12-hour period.

a. EXPERIMENTAL METHOD

I. Outdoor Cultures - All three sizes of tanks (A, B and C) were used in these experiments. There were two kinds of experiments possible: 1) everything else, being the same

(i.e., inoculum size, total volume, nutrient composition, other than sugars, etc.) a series of the same-sized tanks could be used to obtain the effects of added molasses or sugars, and the effects of the modes and times of addition. 2) the effect of addition of organic carbon, when all variables e.g., size of inoculum and tanks, concentration of nutrient, capacity of tank, etc., were considered. Both classes of experiments are reported here. The usual mode of addition of molasses was to add it at a concentration in the range of 0.06 to 0.2 g/l at about 5-7 p.m. in the evening. Molasses from Mysore Sugar Mills, Mandya, Mysore, containing 42% reducing sugar was used. Later analysis reveals that molasses does not contribute to the growth (per se) at night. However, this was contrary to the expected results of the experiments. In view of the expectation, no molasses additions were undertaken at day time.

The analysis of the sugar content in the medium was carried out using the phenol sulphuric acid (Dubois et al., 1956) with a standard calibration curve (Appendix 5.1) on the centrifuged clear liquid. The carbohydrate in algal biomass was determined by the method given in Appendix (5.2) using anthrone reagent. Microscopic examination for bacterial contamination was undertaken at intervals.

A tacit assumption made in this chapter is that optical density versus dry weight follows the same calibration curve (Figure 4.1.1) with or without addition of organic carbon.

II. Indoor Cultures - In tube cultures, indoors three types of treatments tried were: 1) molasses added at the beginning of the dark cycle, 2) molasses added at the beginning of the light cycle, and 3) molasses added in 4 doses at 6 hourly intervals. Air was bubbled through the tubes, but no extra carbon dioxide. Optical density of the cultures was measured at the beginning of the light and dark cycles. Experiments were carried out in duplicate.

b. RESULTS

I. Outdoor Cultures - Figure 5.1.1 represents the growth (O.D.) versus time for one series of experiments where a set of control, control plus molasses, control plus carbon dioxide runs were made at the same time of year under identical outdoor conditions. In all tanks inoculum size, total volume, and tank size were constant. Table 5.1.1 shows the difference in algal carbohydrate for one run between morning succeeding evening, next morning and so on when molasses was added.

Bacterial contamination was never significant, apparently due to the rather low sugar concentrations. Protazoa infection which took place occasionally at higher algae concentration was minimized by lowering the pH and raising it again after 2 hours.

Figure 5.1.2 represents the dissolved oxygen, pH and light conditions for one set of mixotrophy-runs. Figure 5.1.3 represents a semilogarthimic plot of about 40 data points (tabulated in Appendix 7) of ratio of sugar to original sugar

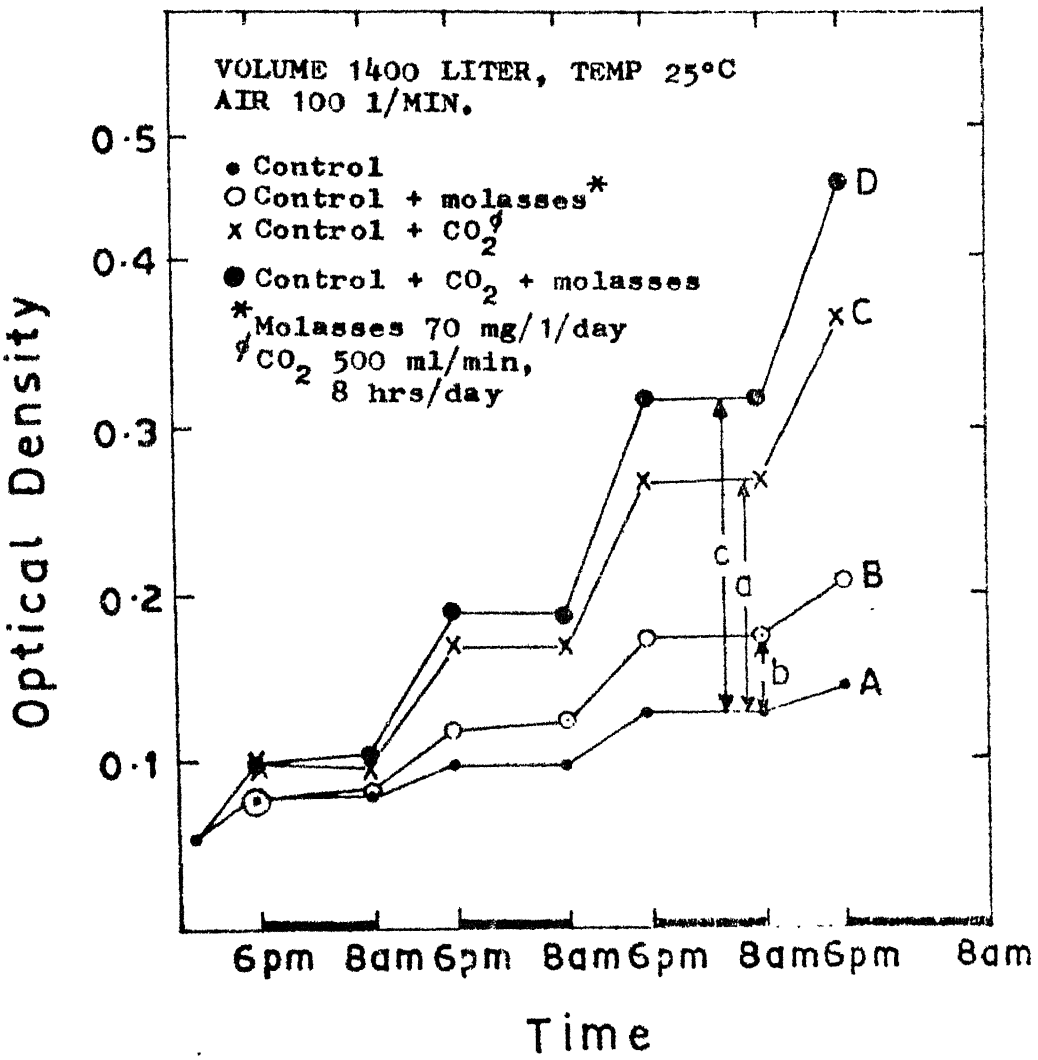

FIGURE 5·1·1 GROWTH OF ALGAE IN OUTDOOR TANKS

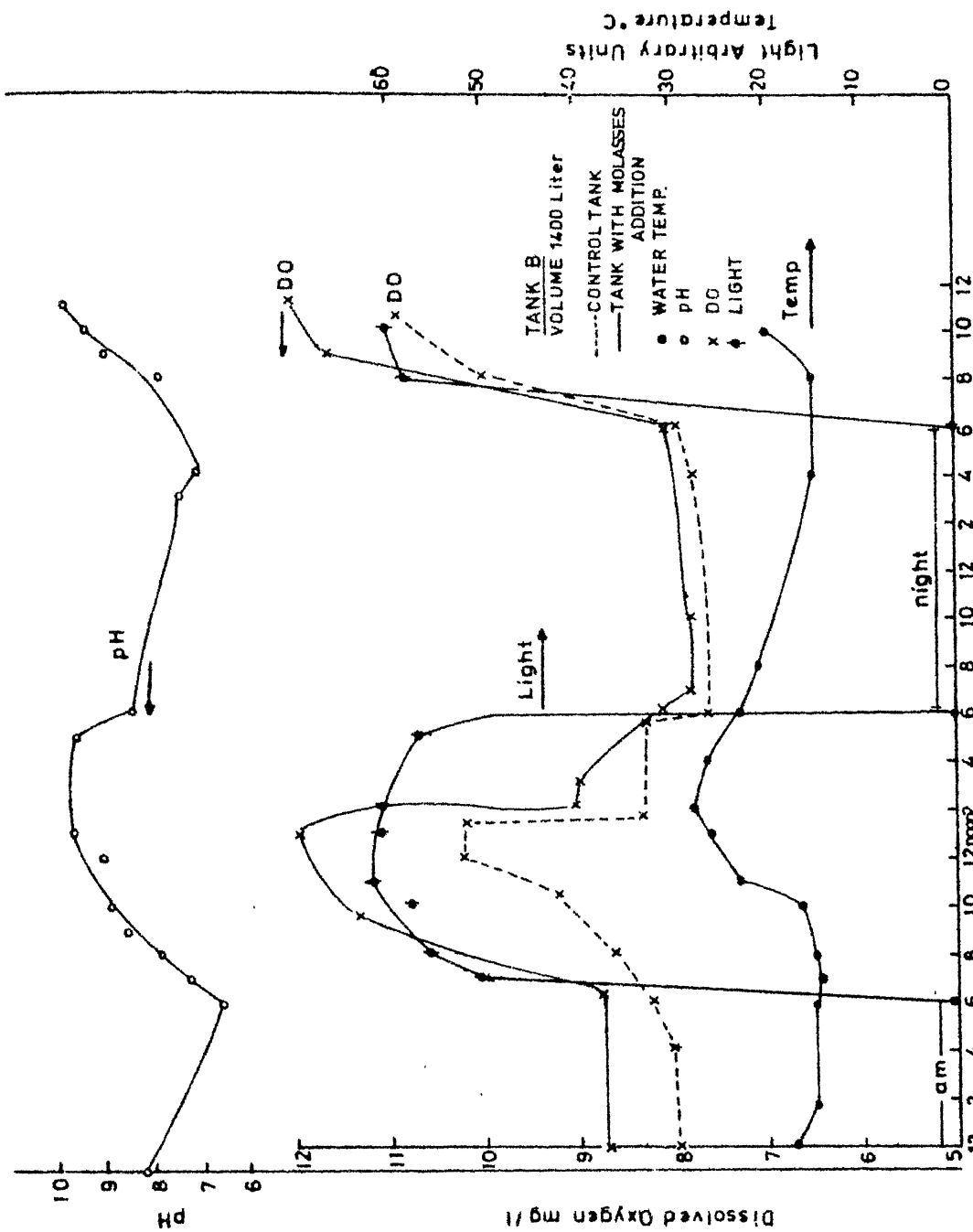

FIGURE. 5·1·2 TEMPERATURE, DISSOLVED OXYGEN AND SOLAR RADIATION IN MIXOTROPHIC AND AUTOTROPHIC TANKS

(in medium) versus time multiplied by optical density. The data points of Figure (5.1.3) are with the addition of molasses, pure glucose, outdoor cultures in A, B and C tanks, with various initial inoculum concentration, and with a range of sugar additions, over the period September 1975 to December 1975.

TABLE 5.1.1

SUGAR % IN ALGAE IN THE MORNING AFTER SUGAR ABSORPTION IN DARK, AND IN THE EVENING AFTER PHOTOSYNTHESIS

Morning Sugar %	Evening Sugar %
8.7	6.0
8.8	6.1
9.3	6.6

II. Indoor Cultures - Figure 5.1.4 shows the optical density at the beginning of the light and dark cycles. These curves do not show a regular pattern of non-usage of molasses during night; in fact the optical density some times decreases during night. There is no explanation for this observation. The addition of molasses in intermittent mode leads to a significant increase in growth over either morning or evening additions when added only once a day.

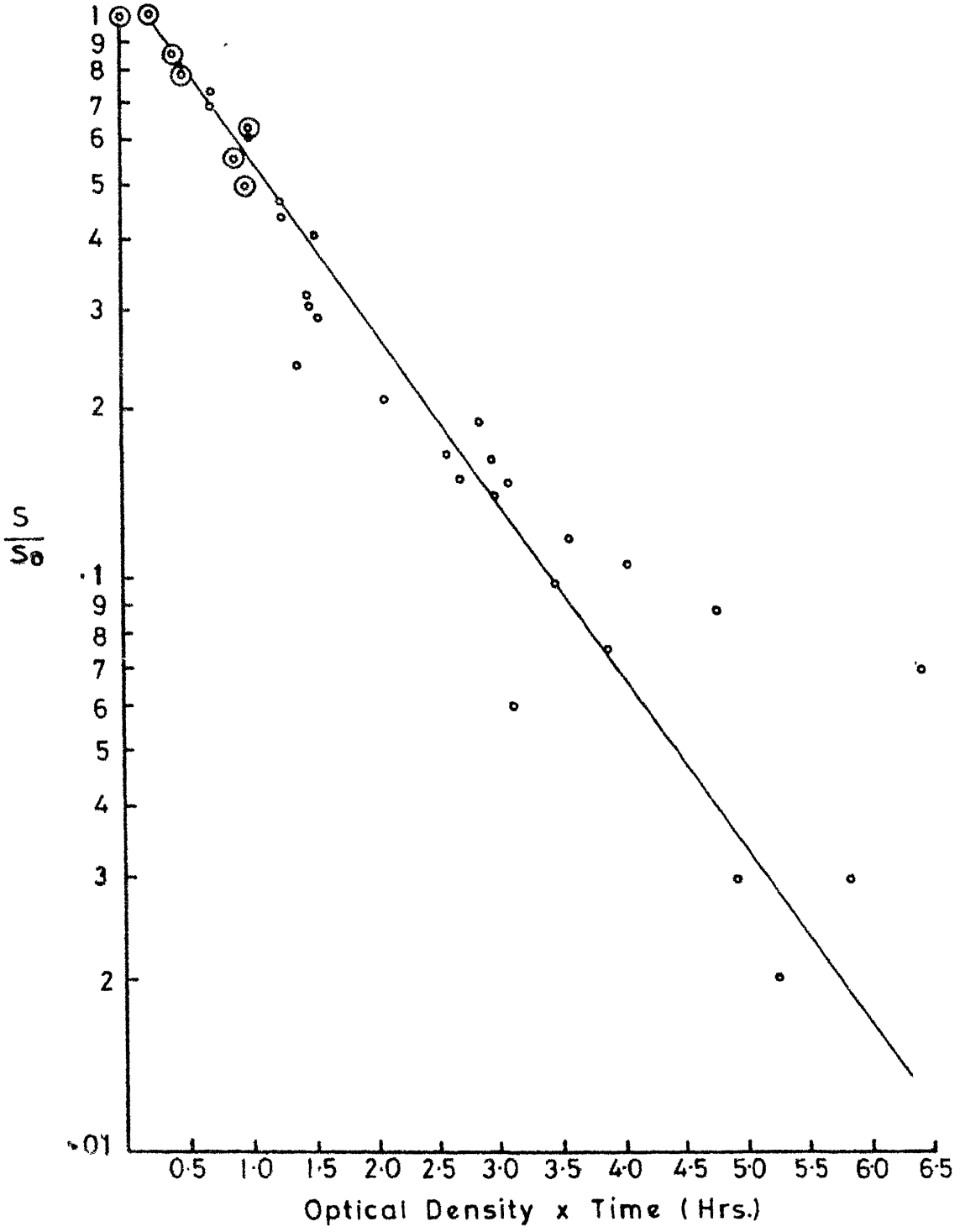

FIGURE. 5·1·3 S/So VERSUS OPTICAL DENSITY x TIME

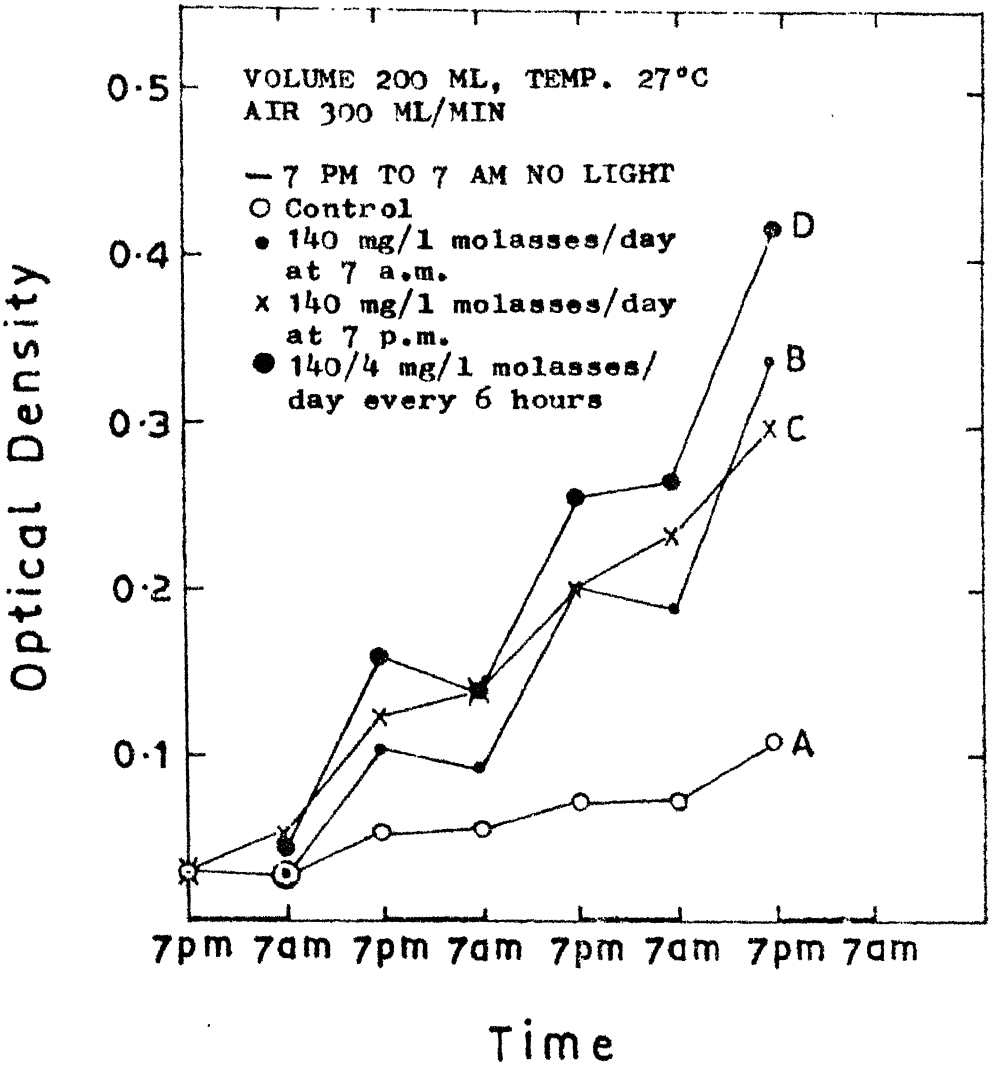

FIGURE 5·1·4 GROWTH OF ALGAE UNDER DIFFERENT MODES OF MOLASES FEEDING (Indoor culture)

c. DISCUSSION

I. Outdoor Cultures - Though it was thought following Endo (1972) and Ogawa (1972), that algae can grow in the absence of light, using organic carbon, these experiments do not bear this out. Figure 5.1.1 shows that during the night there is no gain in algal dry mass (as measured by optical density). On the other hand, the algae dry mass seems to absorb the carbohydrate in the course of the night and utilise it during the day time. This then leads to an increase in growth over the control experiment without carbohydrate. One possible explanation for the lack of growth during night is that the algae were not dark-adapted for growth as were Ogawa's (1972) culture. Since our cultures were grown in the laboratory under photosynthetic conditions and then used as inoculum, these cultures never adapted for growth during night. It seems possible however, that during periods of low light intensity e.g., during the monsoon, some dark adaptation to organic carbon would result in higher yields. This idea needs to be developed. The ideal of mixotrophy, i.e., phototrophic growth by day and organotrophy by night has not been achieved here; nevertheless higher biomass production has been obtained.

Figure (5.1.1) shows that for the addition of molasses of 0.07 g/l per night for 3 nights there is a net increase of 200% over control when carbon dioxide and molasses are used, 150% over control when only carbon dioxide is used, and 48% over control when only molasses is used. This is over a 4 day

period and growth is about 17 $g/m^2/day$ for the best conditions used here (carbon dioxide + molasses). A range of molasses concentrations much wider than used here would be necessary to arrive at the optimal concentration of molasses for best yield of algal biomass. However, due to the danger of bacterial infection such a wide range was not tried. It may be noticed that the effect of carbon dioxide and molasses addition is cumulative i.e., equal to the sum of the effect of carbon dioxide alone and the effect of molasses alone. From Figur 5.1.1 the following table (5.1.2) is obtained.

TABLE 5.1.2

INCREASE OF ALGAL BIOMASS PER 24 HOUR PERIOD DUE TO AIR, AIR + CARBON DIOXIDE, AIR + MOLASSES AND AIR + CARBON DIOXIDE + MOLASSES

(O.D. SCALE)

	Air + molasses - air	Air + carbon dioxide - air	Air + carbon dioxide + molasses - air
	1	2	3
0th evening	0	0.02	0.02
1st evening	0.02	0.07	0.09
2nd evening	0.045	0.140	0.190
3rd evening	0.075	0.225	0.305

The table shows that the sum of columns of 1 and 2 add upto column 3. The range of carbon dioxide and molasses

concentration wherein this effect is present or optimal is not known. From the data, it may be said that for this range the effect of added sugar is cumulative to the effect of added carbon dioxide, inspite of the different metabolic pathways for the utilisation of these substrates. Table 5.1.1 shows the difference in algal carbohydrate (Anthrone method, Appendix 5.2) in the morning after molasses absorption in the dark, and in the evening after photosynthesis. The material balance on the algal carbon (assuming 50% carbon in algae) and the molasses carbohydrate-carbon is not satisfied using the anthrone method. However, the table does show that sugar is absorbed in the dark, and used in the day.

The alternate method to obtain a carbon balance is to use the growth during one day, subtract out that due to photosynthesis and check whether 50% of the residual algae is due to the added molasses. By this method, many experiments give an average of 95% carbon utilisation of the molasses-carbon (reducing sugars). The remainder of the molasses-carbon is not accounted for.

Some of the points to note from figure 5.1.2 are as follows:

1. Temperature started decreasing since 2 p.m. (25°) and went down to 15° at 4 a.m.

2. pH of the culture started decreasing from 9.6 at 5 p.m. to 7 at 4 a.m. This decrease in pH was probably due to the

low desorption rate of carbon dioxide at low temperature. Non-increase of pH in night supports that there is no growth in night in cultures with molasses.

Dissolved oxygen concentration in the tank with molasses in this particular run was higher than the dissolved oxygen in blank tank suggesting that the addition of molasses does not increase the respiration rate of algae. Some times the dissolved oxygen in tanks with molasses were found lower e.g., in one run: dissolved oxygen of water was 5.8 mg/l, dissolved oxygen of water saturated with air was 7.86 mg/l, in tank C (with 50 gm glucose added at 6 p.m., optical density 0.13) it was 6.9 mg/l, in tank C (without molasses, optical density 0.135) it was 7.6 mg/l. This leads to the conclusion that oxygen is not limiting in molasses fed cultures in night.

II. Indoor Cultures - Indoor cultures show much more yield with intermittent feeding of molasses i.e., feeding molasses in 4 dumps. This result of indoor intermittent feeding supports the above hypothesis that absorption of sugars is not a rate limiting step. In Chapter 8, the intermittent feeding of carbon dioxide is discussed. Intermittent feeding of carbon dioxide also leads to increase in growth rate. Perhaps intermittent feeding of nutrients leads to a condition similar to synchronous cultures, which gives higher growth rates of algae. The increase in yield over the control at the end of 3 days by

adding 23 mg molasses into 200 ml culture per day was 209% when molasses was added in the morning, 172% when molasses was added in the evening and 286% when molasses was added in 4 dumps.

These results are of considerable significance for algal effluent treatment systems. Intermittent effluent release will lead to improvement in treatment efficiency.

d. ABSORPTION KINETICS

The plot of ratio of initial sugar concentration to sugar concentration at any time t on log scale against the product of optical density and time in hours (Fig. 5.1.3) is a straight line. The scatter of data on lower right hand side of the graph is due to the error in estimating very low concentration of sugar in algal cultures. This and the fact that the increase in growth of algae due to molasses is in day time leads to the hypothesis that absorption of sugar follows the following reaction kinetics during the night:

$$Ag + S \longrightarrow AgS \qquad \ldots\ldots (5.1)$$

where, Ag is algae, S is sugar, and AgS represents algae in which sugar is stored. The absorption of sugar by algae is a function of surface area available for mass transfer and difference in sugar concentration outside and inside the algae.

Let us assume the transport of sugar to the algal cell to be active transport, i.e. a transport which utilizes A.T.P. to overcome the concentration gradient and is independent of

the concentration inside the cell. Taylor (1960) found that the transport of glucose into the algae is active transport. By this assumption the rate of absorption ofsugar by algae at night time can be represented by:

$$-\frac{dS}{dt} = K a S \quad \ldots\ldots \quad (5.2)$$

where, S = concentration of sugar in medium at time t, a = area available for mass transfer and k = rate constant. But area available for sugar transfer 'a' is proportional to the number of cells or the dry weight of algae per unit volume.

$$-\frac{dS}{dt} = K' Ag S \quad \ldots\ldots (5.3)$$

where, Ka = K'Ag and Ag = concentration of algae in culture. Integrating equation (5.3),where Ag is a constant at night-time:

$$-\ln \frac{S}{So} = K' Ag t = K^* x t \quad \ldots.(5.4)$$

where, So is concentration of sugar in algal culture at time t = 0, and x is absorbance of the culture. The value of K', calculated from Figure (5.1.3) was found to be 0.68 1/g.hr. At an optical density of 0.4, 90% ofthe sugar will be absorbed in about 9 hours, and at the same optical density 50% will be absorbed in three hours.

From the above discussion it is clear that the growth of algae due to organic carbon is made of two processes; 1. The absorption of sugars and 2) the synthesis. Negligible growth in night in presence of organic carbon suggests that

either the synthesis process is slower than absorption of sugar, and is a rate limiting step or requires light for algal cell synthesis. This suggests that the organic carbon can be added at any time into the culture without making any difference in gain of algae, since the absorption of sugar is very rapid. The above conclusion can be extended to postulate that the growth and carbon utilization efficiency can be increased by adding the organic carbon intermittently. The increase in growth of algae by intermittent addition of molasses are reported (Seshadri and Misra 1977).

e. CONCLUSIONS -

Following are the major conclusions from this Chapter:

1. The absorption of sugar into the algal biomass is much more rapid than its utilisation for cell mass synthesis.

2. There seems to be no growth as such at night time, instead the absorbed carbohydrates are used in the day time.

3. At low concentration of molasses the effect of carbon dioxide and molasses addition is cumulative.

4. The addition of molasses intermittingly increases the growth significantly.

5. 95% of carbon goes into the algae. Rest 5% is probably lost in respiration, etc. This leads to the conclusion that addition of molasses does not increase respiration rate of Scenedesmus acutus.

6. The absorption of sugars in night follows a first order kinetics with respect to sugar, and concentration of algae as a parameter.

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CHAPTER 6

MIXING PARAMETERS

In Chapter 4 the effect of mixing and aeration on growth was discussed. There the conclusions are:

1. Mixing increases the growth of algae by 54% over the unmixed cultures.
2. Aeration alone increases the growth by 15.4%.
3. There is no increase in growth by aeration when added over mixi
4. The mixing by paddle wheel is sufficient to maintain aerobic conditions in night in algal cultures.

In this section the effect of hydrodynamics of the tanks on mixing is discussed.

To carry out a complete mixing analysis and experiments a sophisticated instrument such as a hot film anemometer is necessary for the measurement of velocities and shear stresses. Since this was not available, the flow parameters and a velocity curve arrived at indirectly from tracer experiments.

A mixing parameter of importance to algal studies is the dispersion coefficient. The determination of longitudinal dispersion is necessary to obtain an estimate of vertical mixing and mass transfer coefficients for aeration and absorption of carbon dioxide.

In dense algal cultures, it is necessary that all the cells be exposed to light uniformly. Hence an idea of how frequently cells from the bottom are brought to the surface will be useful. The best way to do this would be to inject known amounts of tagged particles at the bottom of the vessel and to do in situ sensing of the particles concentrations at various heights. Such a sensitive detector was not available. Hence the studies here have not touched this important area. Mass transfer and dispersion coefficients have been determined however.

The classical analysis of Taylor (1953) for pipe flow has been extended by several authors to open channel flow. It is not clear that many of these equations can be applied to the present system because, here a paddle wheel was the source of energy (not gravity), and the amount of energy so delivered could not be measured. Morever the depth of immersion of each paddle (unknown) strongly influenced the velocity and other dependent parameters of the system. However, an attempt has been made to correlate some of the hydrodynamic parameters using published information and to check whether experimental data and calculated values are consistent.

a. EXPERIMENTAL METHODS

Experiments were performed in all the three sizes of tanks with different liquid volumes. A measured quantity of water was taken in the tank and the paddle wheel started. After the flow was steady, a known amount of methylene blue was injected

An attempt was made to find out the electrical power consumed by the motor for different volumes of the liquid, but there was very little change in the power consumed, and it was difficult to measure the change. A pitot-tube was used to measure the velocity profile in the tank. This measurement of velocity profile was not possible due to atmospheric wind-induced vibrations.

The following were the sources of error:

1. The velocity measurements were indirect (from dye studies); though the open-channel flow theory may be extended to the straight portions of the channel, the curved portions (even though baffles were provided) did bring about some cross-sectional homogeneity in mixing of the tracer. Hence the error in velocity measurement due to mixing at curved portions will provide some departure from theory.

2. The velocity at the surface of the tank was non-uniform especially where the paddle wheels provided considerable churning and turbulence. Also longitudinal waves were quite pronounced especially in C tanks.

3. There was considerable back mixing of surface particles in the region of the paddle-wheels.

b. RESULTS

The results are plotted in the sample curves, Figures 6.1, 6.2 and 6.3 for A, B and C tanks. The tabulated data

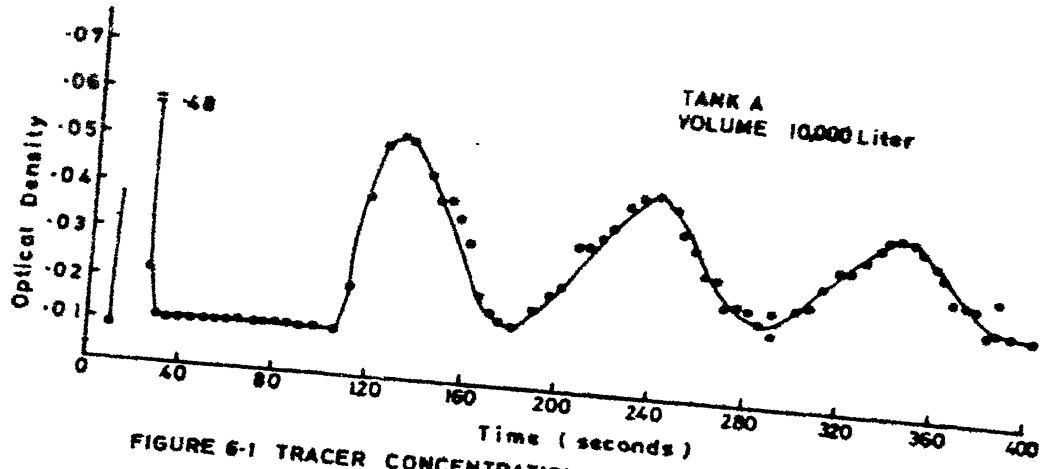

FIGURE 6-1 TRACER CONCENTRATION VERSUS TIME CURVE

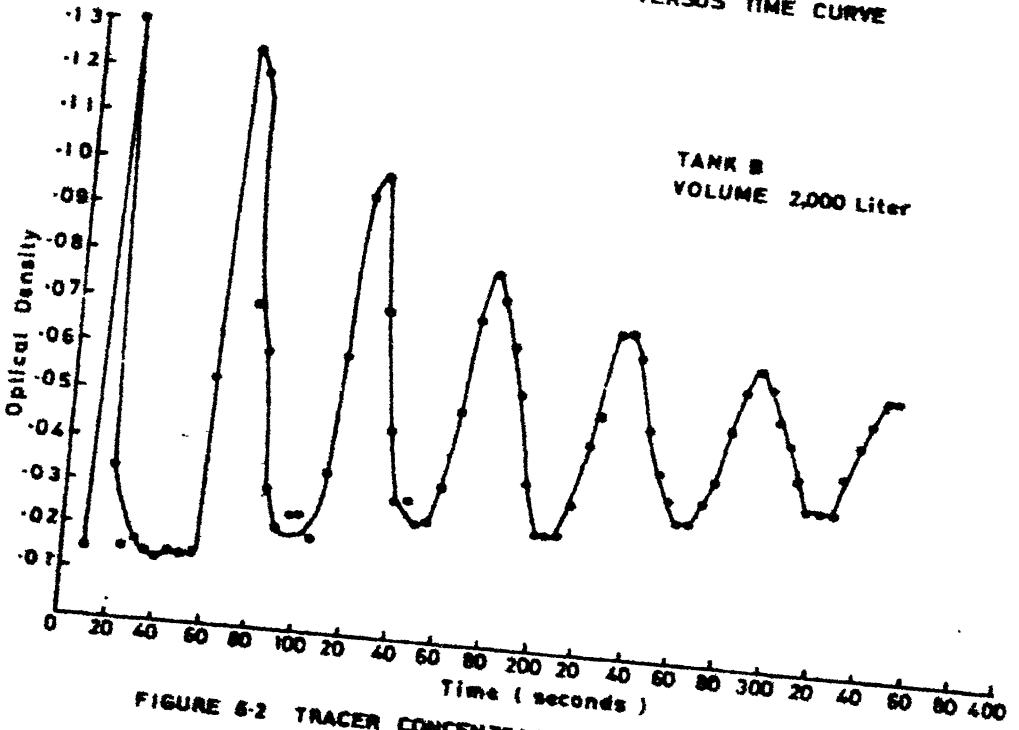

FIGURE 6-2 TRACER CONCENTRATION YERSES TIME CURVE

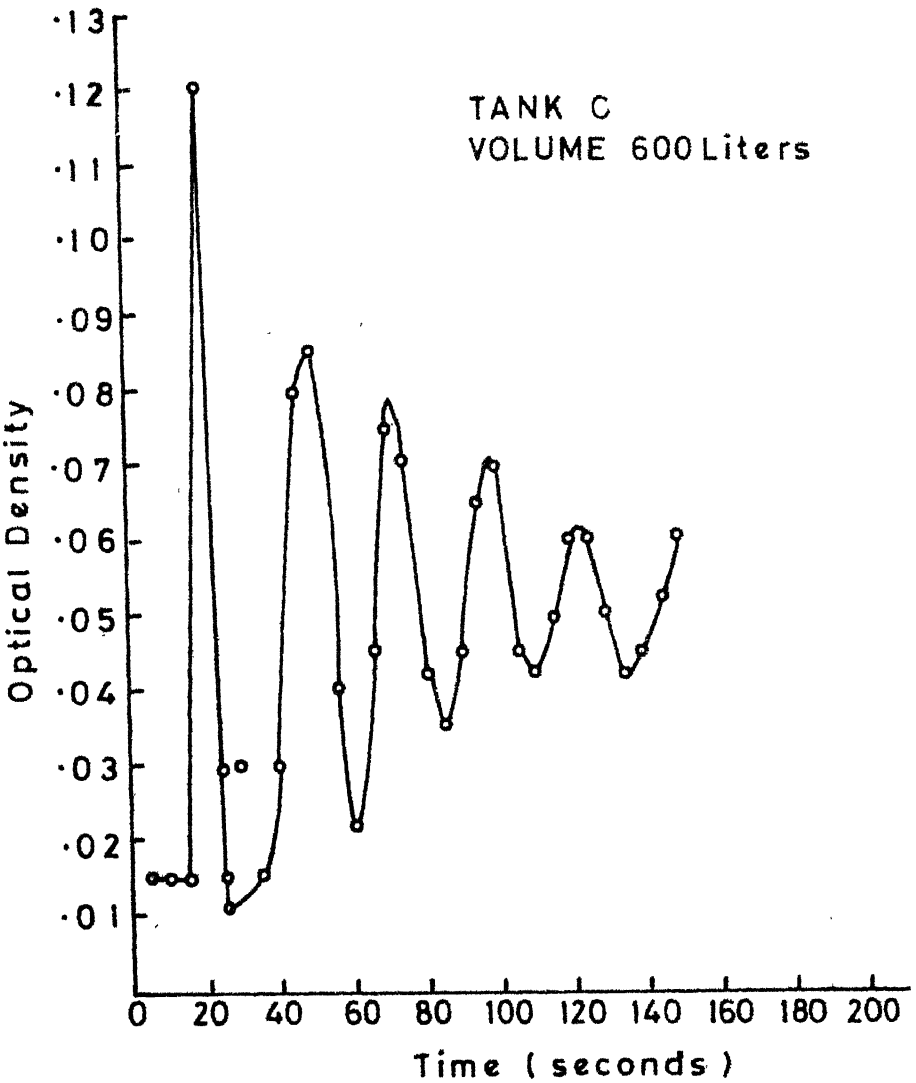

FIGURE 6·3 TRACER CONCENTRATION VERSUS TIME CURVE

are given in Table 6.1, which gives all the derived results from this section and the next.

Some important points to be noted are as follows:

1. The velocity increases with increase in volume in the tank due to more of the area of the paddle being effective in pushing the fluid.

2. The curves of Figures 6.1, 6.2 and 6.3 are almost symmetrical after the first wave of skew distributed concentration.

3. The algal culture experiments usually lasted six days. Hence the question of mixing of liquid nutrients is not important because in most cases the dye was well mixed after about 15 minutes even at the lowest velocity.

c. THEORY AND DISCUSSION

The literature has been well reviewed by Fischer (1973). In the treatment that follows, some crude assumptions have been made to arrive at the dispersion coefficient; a regorous analysis and experimental set-up for the dispersion coefficient would involve sophisticated measurements of the hydrodynamic parameters. These measurements were not possible here.

In a strictly two-dimensional open channel flow the longitudinal mixing of a tracer into the stream can be, (following G.I. Taylor) expressed through a unique coefficient

TABLE 6.1 TANK PARAMETER AND DIMENSIONLESS NUMBERS

Tank:	Volume liter	h cm	D_L cm^2/sec	$\bar{u}$ cm/sec	R_H cm	P_e /	Re , 10^2	k 1/sec	k_L cm/sec	S_H 10^5	D_L/h^2 1/sec
A	6000	11.4	1017	44	37.9	1.64	1667	0.00019	0.0022	0.043	7.82
W =	7000	13.2	1681	45.5	42.7	1.15	1943	0.00029	0.0038	0.08	9.64
108 cm	8000	15.1	2135	49.2	47.5	1.09	2342	0.0002	0.003	0.056	9.36
L = 227.0cm	10000	18.7	2312	46	56.1	1.16	2785	0.00013	0.0024	0.07	6.6
B	800	7.76	190	24.7	24.1	3.133	595	0.00038	0.0029	0.0378	3.15
W=54 cm	1000	9.66	250	26.4	28.46	3.00	751	0.00031	0.0029	0.042	2.68
	1200	11.5	375	30.9	32.3	2.66	998	0.00026	0.0029	0.049	2.8
L =	1400	13.31	350	32.4	35.66	3.3	1155	0.0003	0.0039	0.072	1.97
892 cm	1600	15.06	412	32.4	38.7	3.0	1254	0.00017	0.0026	0.052	1.82
	1800	16.8	573	35	41.4	2.82	1449	0.00017	0.0028	0.054	1.8
	2000	18.5	735	35.6	43.9	2.13	1564	0.00016	0.0029	0.068	2.1
C	500	12.45	581	31	34.1	1.82	1056	0.00046	0.0057	0.1	3.7
L =	600	14.8	693	38.8	28.8	2.14	1482	0.00044	0.0066	0.089	3.16
290 cm	700	17.1	764	38.8	41.9	2.12	1624	0.00034	0.0058	0.007	2.6
W=54 cm											

where, $Pe = R_H \bar{u} / D_L$; $R_e = R_H \bar{u} / \nu$ and $S_H = k_L R_H / \mathcal{D}$

of dispersion. This coefficient of dispersion can then be related to the mass transfer coefficient at the surface for absorption of oxygen or desorption of carbon dioxide. In a three-dimensional flow, eddy coefficients in each direction have to be considered for arriving at transfer coefficients and mixing rates. Hence for a first approximation a two-dimensional model is useful. A different attack on the problem is to base the calculation of mass transfer on a surface renewal mechanism (Wadkarni and Russell 1973). This is an empirical treatment and has less claim to soundness.

ASSUMPTIONS

The assumptions made here are used to justify the two-dimensionality and to apply the equations in the literature.

1. The flow can be approximated by two-dimensional flow because the boundary layers on the sides of the tanks are much thinner than the width or the height of the flowing fluid; $w/h \gg 1$; $L/w \gg 1$; where w is the width of the tank, h the height of liquid and L the length. The curved portions have baffles which minimise the effect of curvature on mixing.

2. The measurement at the same stations of concentration profiles with time, but separated in time by one circuit of the tanks, gives what would in a normal open channel flow, be the measurement at different stations; i.e. instead of x_1 and x_2 where x_1, x_2 are two longitudinal stations at which the concentration time profiles are obtained, it is proposed to

use $\bar{u}t_1$ and $\bar{u}t_2$ where $\bar{u}$ is the mean flow velocity and t_1, t_2 are the times taken to traverse two successive circuits of the tank.

3. The injection of tracer at the point of average velocity 0.6 h, and subsequent sampling at the same point is equivalent to injection of a line source, for purposes of obtaining an apparent coefficient of dispersion. The reasonableness of this assumption is discussed by Chatwin (1972).

4. The concentration time curves are normally distributed (as shown in Figure 6.1) after an initial period where the skewness is pronounced. This (normally distributed) period is after the convective period where the diffusion equations of two-dimension flow are not applicable (Fischer, 1968). After this initial period the normality of the curves allows us to equate the time of travel of the diffusive cloud to the average flow velocity times the distance travelled (see Thackston, Hays and Krenkel 1967).

A number of ways of arriving at the dispersion coefficient D_L are reviewed by Thakston, Hays and Krenkel (1967). Of the methods given the method of Fisher (1964) and Aris (1956) was chosen here as being the most convenient. This is given as:

$$D_L = \frac{\sigma_{t_2}^2 - \sigma_{t_1}^2}{2\Delta t} \bar{u}^2 \qquad \ldots (6.2)$$

Where , D_L = Dispersion coefficient

σ_t^2 = Variance of the time concentration curve for circuit 2 and 1, (see equation 6.1)

Δt = Average time for one circuit

$\bar{u}$ = Average velocity of liquid in tank.

These values are given in Table 6.1 and are in agreement with published values in laboratory channels, (Thackston, Hays and Krenkel 1969). The formulae that have been derived relating D_L to other parameters such as friction velocity, U_*, could not be verified here, vertical and transverse mixing coefficients have been also predicted using the friction velocity. However, a useful prediction using the dispersion coefficient is:

$$k_2 = \alpha \frac{D_L}{h^2} \qquad \ldots \qquad (6.3)$$

where, k_2 = Reaeration coefficient

D_L = Dispersion coefficient

h = Height of the liquid in tank

α = Constant

This analysis is carried out in the next section.

d. CONCLUSION

On the basis ofthe above discussion and results the conclusions derived are:

1. Average velocity increases with the increase in liquid depth.

2. Dispersion coefficient increases with the increase in liquid height.

3. Reynolds number based on hydraulic diameter is always greater than 595×10^2, this Reynolds number in open channel (gravity flow) is in turbulent flow regime.

4. Peclet number based on hydraulic diameter and dispersion coefficient varies from 1 to 3.3 in most of the cases. For any one tank it is reasonably constant within experimental error.

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CHAPTER 7

CARBON DIOXIDE TRANSFER

Algae contain 50% carbon, which is derived by photosynthesis from carbon dioxide. Carbon dioxide budget is considered as one of the most important problem in designing mass culture equipments. The problem of gas transfer in algal cultures may be divided into three parts:

1. Supply of carbon dioxide to algal cultures.

2. Desorption of carbon dioxide and oxygen to atmosphere through free surface of the culture.

3. Quality, quantity and mode of carbon dioxide feeding.

In this section the decarbonation coefficient (defined later), mass transfer coefficient of carbon dioxide in tap water, and their relationship with hydrodynamics of the tanks are presented. The analysis was done with tap water, because the methods of estimating carbon dioxide in algal suspension available are not reliable. Livanskey et al., (1973) have reported that mass transfer coefficient from water and algal suspension are same. However, the method of estimating carbon dioxide in algal suspension by measuring carbon dioxide concentration in air saturated with sample, uses the same assumption, and is equivalent to measuring the mass transfer coefficient in water.

SUPPLY OF CARBON DIOXIDE

The gas transfer equipments to supply carbon dioxide to algal cultures must be efficient, small in volume and easy to clean. The following authors give a cross section of the type of equipments that have been used.

1. Sparger (Cook, 1951; Leon et al., 1963; and Zuraw et al., 1961).

2. Packed absorption column (Zlokarnik, 1966; Prokeš et al., 1969).

3. Ring shaped hydraulic jump absorber (Smutek et al., 1975).

4. Carbon dioxide in recirculating pipeing and helical casing (Rudas, 1975).

5. Agitated gas liquid bubble contactor (Prokeš et al., 1973).

6. Venturi liquid contactors; (Bauer et al., 1963).

The disadvantage of spargers is that the plate tends to become fouled, and system requires power to force gas through orifice or sintered plate. In packed bed column algae can adhere to the column packing, while spray absorbers may be clogged during extended operation. Ring shaped hydraulic jump absorbers creates turbulence and are reported to be efficient systems. Supply of carbon dioxide into the recirculation piping or into helical casing of the pump were found unsatisfactory (Rudas et al., 1975). The absorption of carbon

dioxide is liquid film resistance controlling, and thus bubble contractors are expected to be more efficient than spray and packed tower. Agitated gas liquid bubble contactors are reported to be more efficient than spray and packed absorption columns. The venturi liquid contactor (Bauer et al., 1963) does not need a compressor since the suction at the throat of venturi provides the necessary deriving force. The limitation of venturi contractor is that it requires low gas liquid ratio for optimum performance.

Carbon Dioxide Measurement

The methods available for measuring carbon dioxide concentration in liquids having suspended solids are not simple and reliable. Due to these difficulties the control and monitor of carbon dioxide in algal cultures have been very difficult. Measuring the carbon dioxide concentration in air in equilibrium with algal cultures for carbon dioxide concentration in liquid is used by Zahradnik (1967), and Livansky et al., (1973). These methods assume that equilibrium concentration of carbon dioxide in water-air and algae-air system is same, which means that equilibrium coefficient is independent of algal and salt concentration. Semenko et al., (1960) have shown that reaching equilibrium even at very high aeration rates is very difficult.

Mass Transfer Coefficient of Carbon Dioxide

In spite of the fact that good amount of work has been done on carbon dioxide transfer, literature on mass transfer

of carbon dioxide from the bubbles of the gas to aqueous solution is not much. Even if available, the results are for situations different from ours. The carbon dioxide transfer process is influenced by solubility and rate of hydration of carbon dioxide, which are affected by pH and temperature of the culture. The present method of feeding the carbon dioxide in our system is not very efficient, and has to be modified. Due to this reason the mass transfer rates from carbon dioxide bubbles to algal cultures were not determined in this study.

However, it was of interest to determine the rate of carbon dioxide desorption from the algal tanks because the leaving carbon dioxide is not available for carbon dioxide budget of algae.

Thus these experiments were carried out with the present spargers which were used to bring the carbon dioxide concentration down to predetermined values in the solutions.

a. EXPERIMENTAL METHODS

1. Carbon Dioxide Supply - In this thesis a simple polyethylene tubes of diameter 2 cm., and with one row of sparger holes at a pitch of 20 cms were used for bubbling carbon dioxide in tanks. One tube in each B and C tank, and two tubes of the same sizes in A tank were used. Algal cultures over pipes were covered by transparent polyethylene sheet, 400 cm long and 75 cm wide to reduce the loss of carbon dioxide to atmosphere.

2. Carbon Dioxide Measurement - In present studies the carbon dioxide in tap water was determined by measuring the pH of the solution. The samples were taken from same depth (0.6 h, where h is height of the liquid in tank) in stoppered sample bottles. Sample bottles were closed in water and pH was determined immediately. A standard plot of carbon dioxide content versus pH was prepared and is shown in Appendix (5.5). Carbon dioxide in tap water was measured according to Deutsche Einheits verfahren Zur Wasser Ab. Wasser Und Schlamm - Untersuchung Appendix (5.5). This method uses the amount of acid and alkali consumed to bring down and raise the pH of the sample to fixed value for carbon dioxide concentration calculations. The above method cannot be used for liquids having suspended solids or dissolved salts. In one of the experiments the amount of 0.04 N sulphuric acid consumed to bring down the pH of the 50 ml of tap water, algae in tap water, tap water + 0.4 g/l urea, tap water + 0.1 g/l urea, and 0.2 g/l algae in urea (0.4 and 0.1 g/l) solution were determined. The volume of 0.04 N sulphuric acid consumed is plotted against pH in Figure 7.1 The Figure 7.1 shows that urea has a buffering capacity and thus the method used in estimating carbon dioxide in tap water cannot be used for solutions containing nutrients and/or algae.

3. Mass Transfer Coefficient - All sizes of tanks were used for the mass transfer studies with different volume of water.

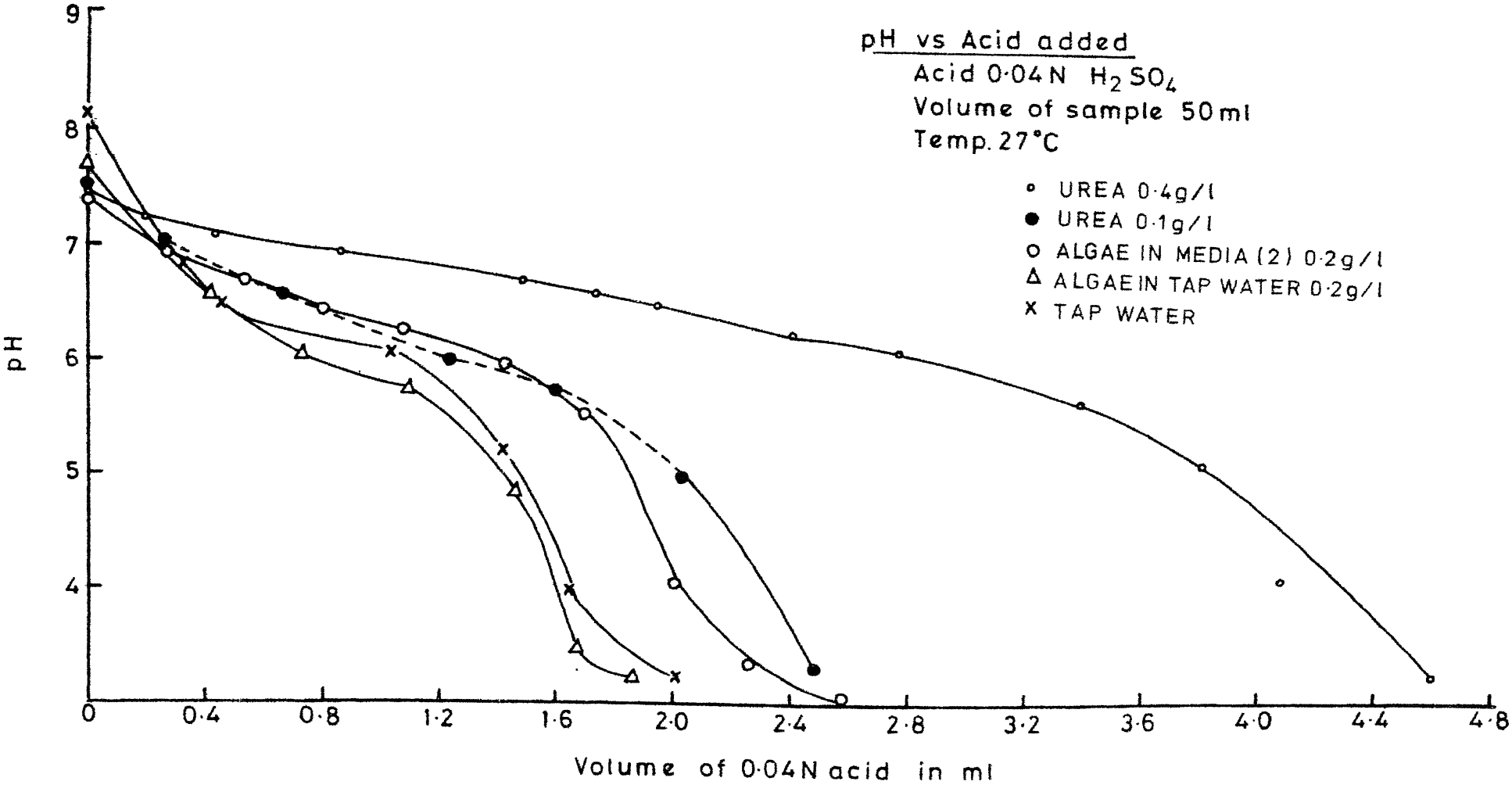


FIGURE 7·1 BUFFERING ACTION OF UREA AND ALGAE

Measured volume of water was taken in the tank, the paddle wheel was started and carbon dioxide was bubbled to saturate the water with carbon dioxide in tanks to pre-determined values. Carbon dioxide was stopped and samples were taken at different intervals of time for pH measurement. Transparent polyethylene sheet was removed at the time of desorption studies. The concentration of carbon dioxide in water was determined from standard pH versus carbon dioxide concentration curve.

In this work, the water in tank is assumed completely mixed and the concentration gradient in longitudinal and transverse direction is ignored. This assumption is justified when the total time which is very high is compared with mixing time and time of one cycle. In completely mixed tank the mass transfer equation can be written :-

$$-\frac{dC}{dt} = k\,(C - Cs) \qquad .....(7.1)$$

Where, C = carbon dioxide concentration in water, Cs = concentration of carbon dioxide in water saturated with air, k = constant = decarbonation coefficient.

Integrating equation (7.1) with initial condition, C = Co at t = 0, gives

$$\ln \frac{(C - Cs)}{(C_o - Cs)} = -kt \qquad ..... \quad (7.2)$$

A plot of $\ln (C - C_s)/ (C_o - C_s)$ versus time would give a straight line of slope k. These are shown in Figure 7.2, 7.3 and 7.4 for A, B, and C tanks.

A liquid film coefficient may be defined by:

$$q = - A k_L (C - Cs) \quad .... \quad (7.3)$$

where, $q = V \frac{dC}{dt}$, V = volume, A = area, and k_L = Liquid film coefficient.

Comparing equations (7.1) and (7.3)

$$k_L = h k \quad ..... \quad (7.4)$$

where, V/A = h = height of the liquid.

Sources of Errors in Carbon Dioxide Mass Transfer Experiments.

The following were the sources of error.

1. The change in concentration of carbon dioxide with pH at low pH values is very high. A small error in pH measurement at low pH may give significant error in carbon dioxide concentration. Carbon dioxide estimation in water should be fast enough to avoid losses of carbon dioxide from sample (which has to be kept mixed for mixing acid or alkali), but the lag in response of pH meter was the limitation in this estimation, and there may be some error due to this lag.

2. The sensitivity of pH meter is 0.1 pH unit, and use of pH meter for a long time does not give stable reading.

3. All hydrodynamic factors effect mass transfer coefficient. The effect of hydrodynamic variability is ignored in this study.

4. The assumption of considering the process as a batch

may not be valid for big tanks.

5. Effect of environmental factors e.g., wind velocity humidity, etc., on mass transfer is not taken into consideration.

6. The flow in C tanks is perpendicular to the flow in A and B tanks, and thus direction of wind in C tanks was always different from A and B tanks.

b. RESULTS

Figures 7.2, 7.3 and 7.4 show that the assumption of considering the desorption process of carbon dioxide as a batch, first order process is quite satisfactory. What is interesting is that in B and A tanks the mass transfer coefficient, k_L is reasonably constant. In C tanks this result does not seem to hold. As has been pointed out, C tanks are very much more turbulent, and therefore may be expected to have higher mass transfer rates.

Table 7.1 gives all the derived results of this section and the previous one.

c. DISCUSSION

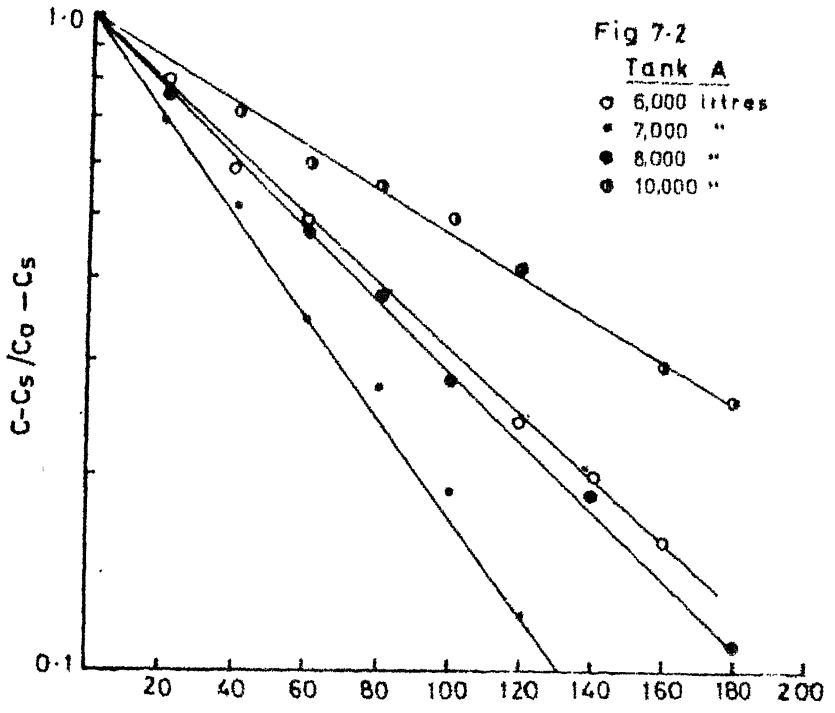
Thakston, Hays and Kronkel (1969) have derived an equation

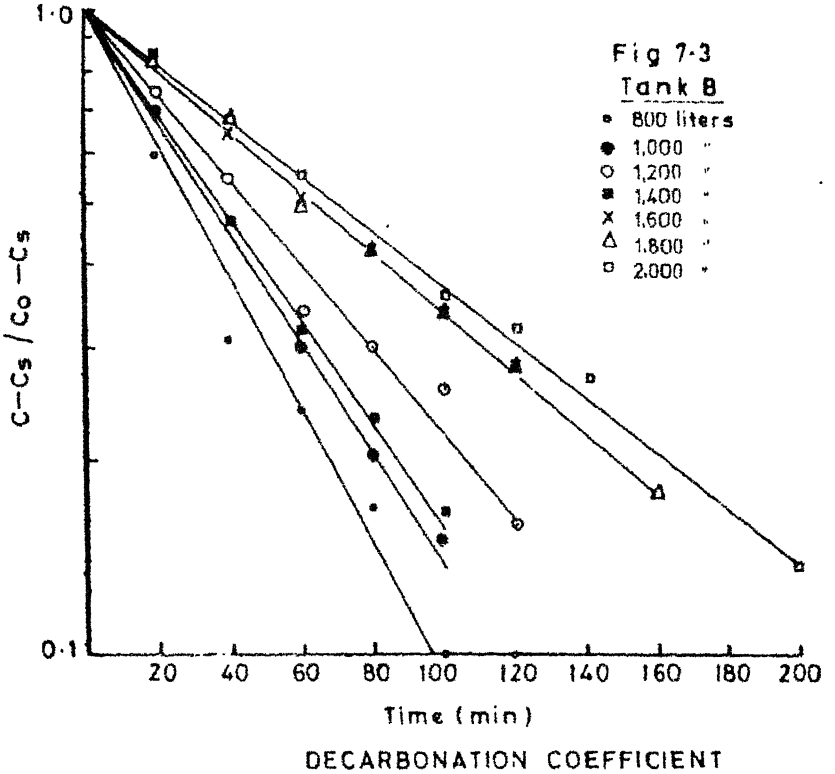
$$k_2 = 0.000015 \frac{D_L}{h^2} \qquad \ldots\ (7.5)$$

based on laboratory and field studies for oxygen reaeration.

where, k_2 = Reaeration coefficient

D_L = Dispersion coefficient, and h the height of the liquid.





DECARBONATION COEFFICIENT

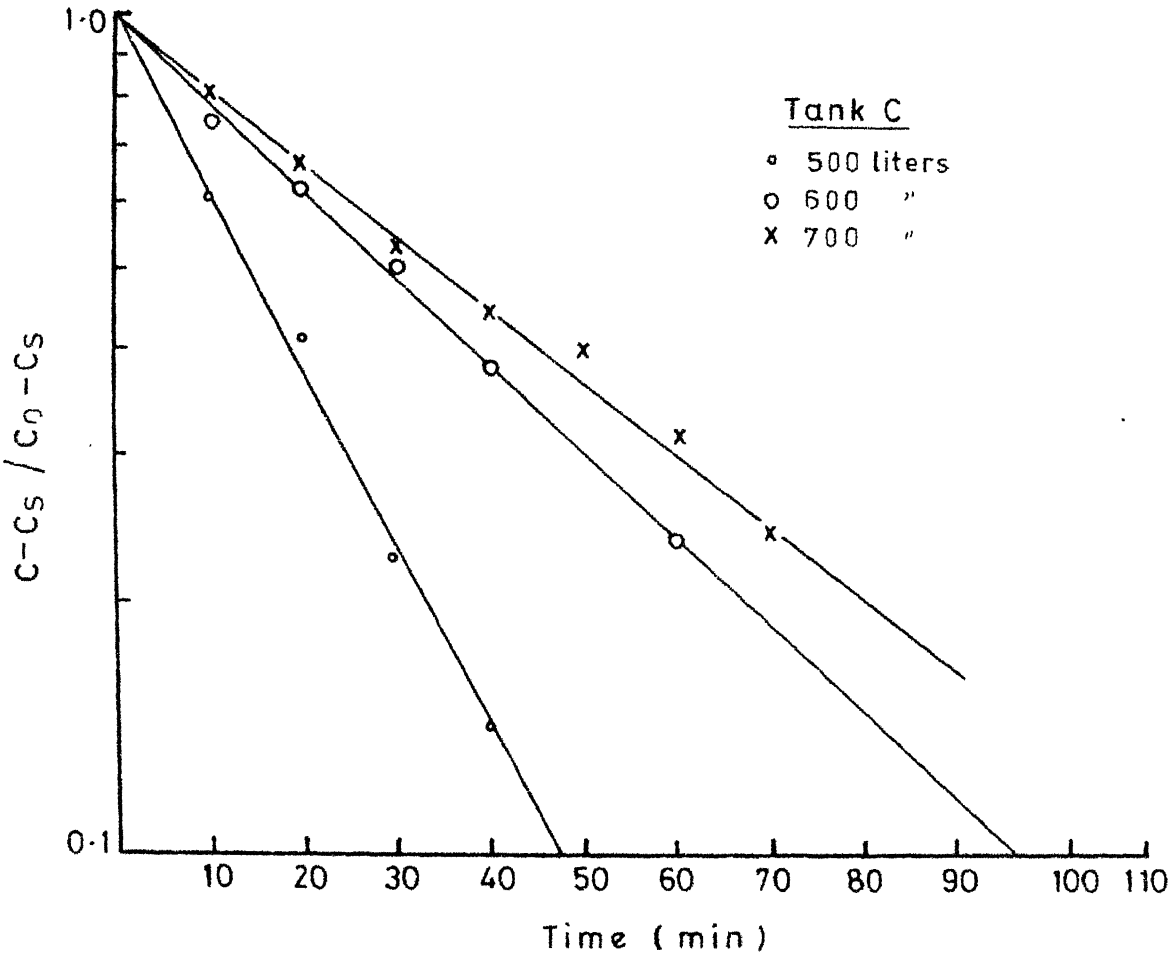


FIGURE 7·4 DECARBONATION COEFFICIENT

TABLE 7.1 TANK PARAMETER AND DIMENSIONLESS NUMBERS

Tank	Volume liter	h cm	D_L cm^2/sec	$\bar{u}$ cm/sec	R_H cm	P_e	Re 10^2	k 1/sec	k_L cm/sec	S_H 10^5	D_L/h^2 1/sec
A	6000	11.4	1017	44	37.9	1.64	1667	0.00019	0.0022	0.043	7.82
W =	7000	13.2	1681	45.5	42.7	1.15	1943	0.00029	0.0038	0.08	9.64
108 cm	8000	15.1	2135	49.2	47.5	1.09	2342	0.0002	0.003	0.056	9.36
L = 2270cm	10000	18.7	2312	46	56.1	1.16	2785	0.00013	0.0024	0.07	6.6
B	800	7.76	190	24.7	24.1	3.133	595	0.00038	0.0029	0.0378	3.15
W=54 cm	1000	9.66	250	26.4	28.46	3.00	751	0.00031	0.0029	0.042	2.68
	1200	11.5	375	30.9	32.3	2.66	998	0.00026	0.0029	0.049	2.8
L = 892 cm	1400	13.31	350	32.4	35.66	3.3	1155	0.0003	0.0039	0.072	1.97
	1600	15.06	412	32.4	38.7	3.0	1254	0.00017	0.0026	0.052	1.82
	1800	16.8	573	35	41.4	2.82	1449	0.00017	0.0028	0.054	1.8
	2000	18.5	735	35.6	43.9	2.13	1564	0.00016	0.0029	0.068	2.1
C	500	12.45	581	31	34.1	1.82	1056	0.00046	0.0057	0.1	3.7
L =	600	14.8	693	38.8	28.8	2.14	1482	0.00044	0.0066	0.089	3.16
290 cm	700	17.1	764	38.8	41.9	2.12	1624	0.00034	0.0058	0.097	2.6
W=54 cm											

where, $Pe = R_H \bar{u} / D_L$; $R_e = R_H \bar{u}/\nu$ and $S_H = k_L R_H/\mathcal{D}$

This equation is based on gravity induced open channel flows. Here this equation has been tested in Figure 7.5 for the decarbonation mass transfer. It may be seen that the line for B and C tanks almost passes through zero. The least square line gave the following equation:

$$k = 1.3 \times 10^{-4} \frac{D_L}{h^2} - 0.23 \times 10^{-4} \quad \ldots (7.6)$$

where k is decarbonation coefficient.
However, in view of the accuracy of the data the line could just as well have passed through the origin. The equation (7.6) is similar for Thackston's (1969) equation.

'A' tank gave a different line of approximately the same slope but a much larger negative intercept, though once again a line passing through the origin would have probably been just as much called for.

Strictly speaking, the mass transfer at the surface does not depend on the dispersion coefficient, since there will be mass transfer even if the fluid velocity is zero. Hence this kind of plot besides checking the existing equations has no basic value.

Nadkarni and Russell (1973) have advanced semiempirical turbulence arguments to arrive at an equation for oxygen transfer in open streams:

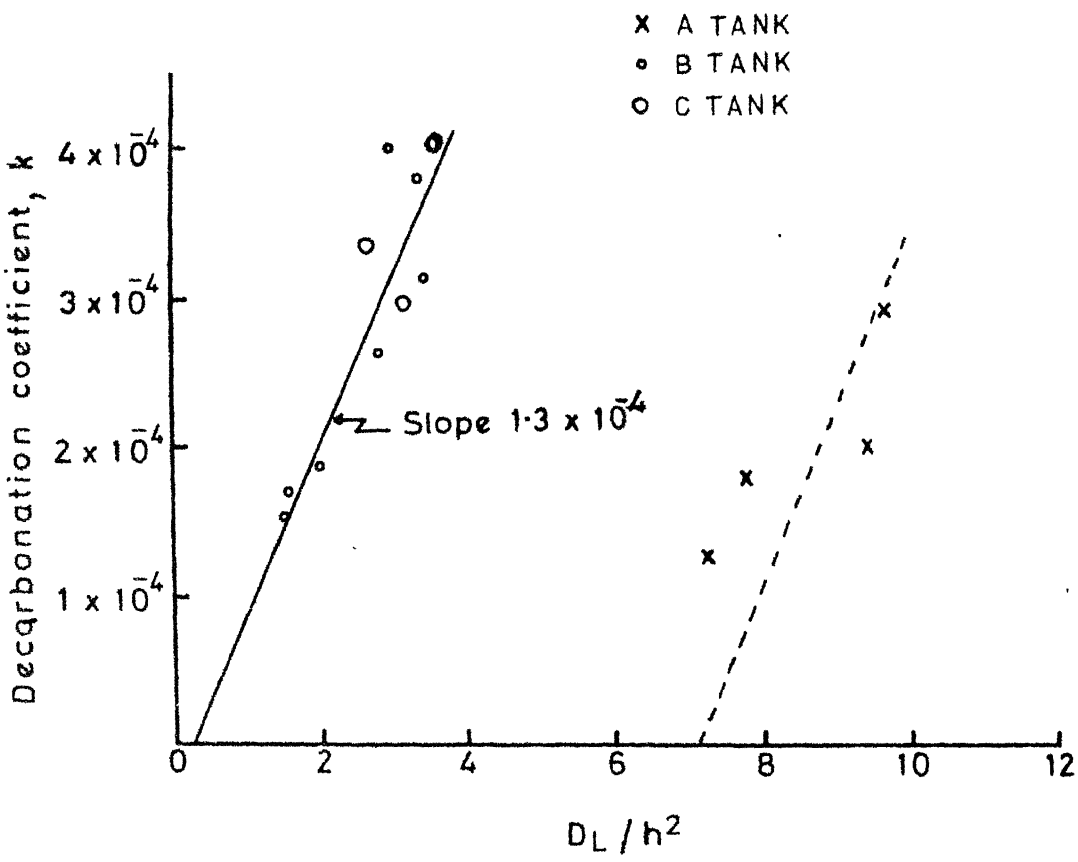


FIGURE 7·5 RELATION OF k and D_L/h^2

$$k_L = 0.651 \quad \sqrt{\nu \mathcal{D}} \; (Re^{0.75}/R_H^{1.08}) \; \phi \; (\frac{h}{R_H}) \quad \ldots \; (7.7)$$

where, k_L = mass transfer coefficient

ν = kinamatic viscosity

$\mathcal{D}$ = diffusivity

Re = Reynolds number

R_H = Hydraulic diameter

h = height of the liquid and

ϕ = functional form of parameters

They used actual data to obtain the functional form of the geometric correction factor ϕ . This equation does not possess any more fundamental significance than the Colburn equation (Rohsenow and Choi 1961). This can be shown as follows:

$$\frac{k_L R_H}{\mathcal{D}} = \beta \; (Sc)^m \; (Re)^n \qquad \ldots\ldots (7.8)$$

where, Sc = Schmidt number

Re = Reynolds number based on R_H

β = constant

or from equation (7.8)

$$k_L = \beta \; \mathcal{D}^{(1-m)} \; \nu^m \; (R_e)^n \; R_H^{-1} \qquad \ldots\ldots (7.9)$$

If m is close to 0.5 and n is 0.8 this equation is identical to Nadkarni's and Russell's equation for a given geometry, where β absorbs the geometric factor.

Here Colburn type equation (7.8) has been tested in the Figure (7.6). The data of the tank B and A can be represented by:

$$Sh = 0.037\ (Re)^{0.83} \qquad \ldots\ (7.9)$$

where, Sh = Sheerwood number = $k_L R_H/\mathcal{D}$

This equation is similar to equation (7.8) where $(Sc)^m$ is hidden in the constant 0.037.

Since k and k_L ($k_L = kh$) are functions of D_L, it was conjectured that D_L should be a function of Reynolds number. This is shown in Figure 7.7. The agreement is fair for a linear dependence on a log-log scale.

d. CONCLUSION

The major conclusions from this chapter are as follows:

1. The desorption of carbon dioxide from tanks can be obtained by assuming it as a first order batch process.

2. Mass transfer coefficient for carbon dioxide from B and A tanks is reasonably constant at 0.0029.

3. Hydrodynamically A, B and C tanks are different. The average velocity of liquid in 'A' tank is much higher than in B and C tanks. The liquid in C tank is much more turbulent than in A and B tanks.

4. Decarbonation coefficient was directly proportional to D_L/h^2 and was related as

$$k = 1.3 \times 10^{-4}\ D_L/h^2 - 0.23 \times 10^{-4}$$

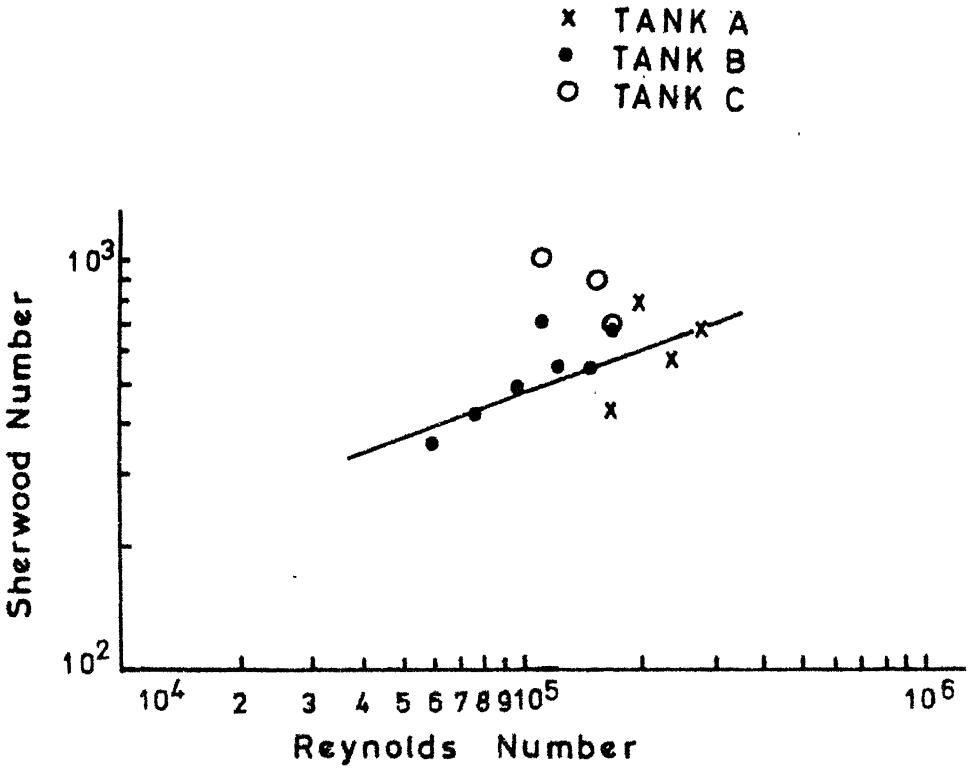


FIGURE 7·6 RELATION BETWEEN SHERWOOD NUMBER AND REYNOLDS NUMBER

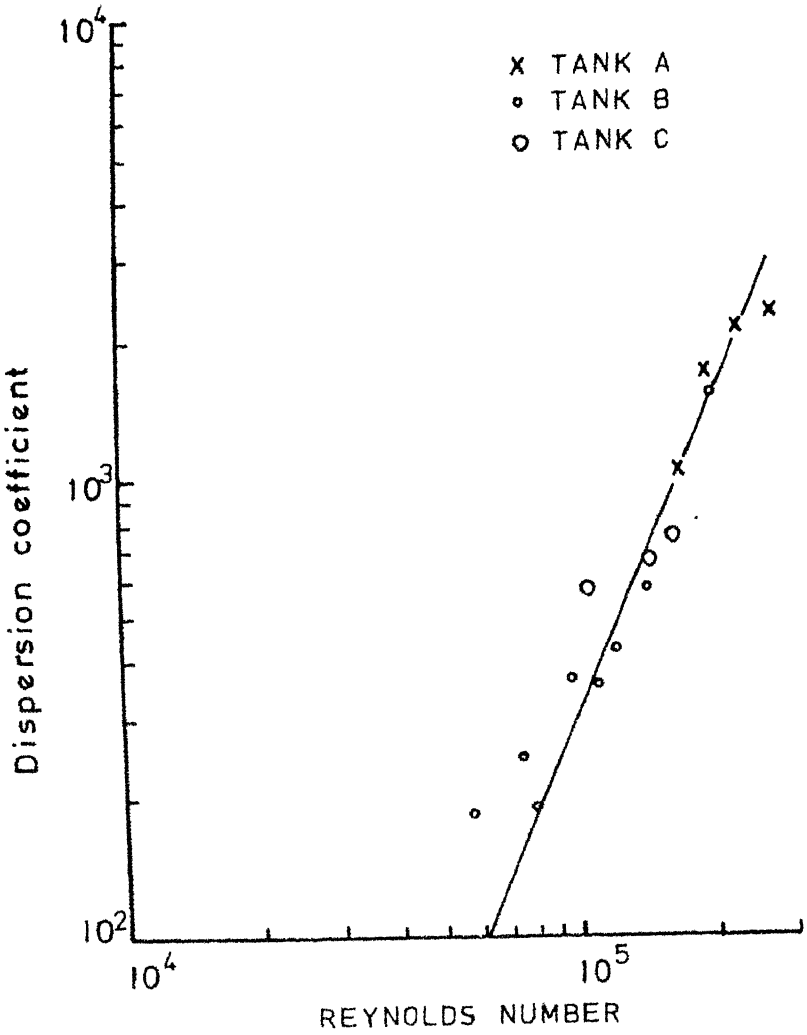


FIGURE 7·7 RELATION BETWEEN Re AND D_L

5. Sherwood number ($k_L R_H/\mathcal{D}$) versus Reynolds number is a straight line of slope 0.83 on log-log plot, and relationship can be expressed as:

$$Sh = 0.037\ Re^{0.83}$$

where, Sh is Sheerwood number and Re is Reynolds number.

6. Dispersion coefficient in tanks was found related to Reynolds number based on hydraulic diameter as:

$$D_L = (1.1 \times 10^{-9})\ (Re)^{2.25}$$

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CHAPTER 8

EFFECT OF CARBON DIOXIDE AND CARBON DIOXIDE FEED METHODS

Algae can utilize carbon from a variety of sources: inorganic carbon (CO_2, HCO_3^-, CO_3^{--}) through photosynthesis, and organic carbon through heterotrophy, or carbon dioxide produced from degradation of organic matter by bacteria. In the systems where other nutrients are in excess e.g., sewage treatment plants, lakes which are eutrophic, and laboratory cultures, it is possible that carbon could be rate limiting. The role of carbon in eutrophication has entered the realm of controversy (Kuentzel, 1970; Sawyer, 1970) and is getting more importance than phosphorus.

If the rate of utilization of carbon dioxide is higher than the supply then the pH of the system would rise. The pH of the culture in turn, affects the distribution of carbon species in the medium e.g., CO_3^{--}, HCO_3^- and CO_2 (see e.g., Stumm and Morgan, 1970). The pH of the medium is coupled with nitrogen assimilation. Every mole of NO_3^- assimilation leads to the production of 1 mole of OH^-, and every mole of NH_4^+ assimilation results in the production of H^+ ion resulting in decrease of pH (Cramer et al., 1948). The role of inorganic carbon on eutrophication is reviewed by Goldman (1972), the effect of inorganic carbon on growth of green algae by Osterlind (1950, 1951), and hydration of carbon dioxide by Kern (1960).

Most of the algal cultures (even fresh water cultures) are carbon dioxide limited (Lange 1967, 1970; Kerr et al., 1970; King, 1971). The supply of carbon dioxide, and the mode of its supply may affect the growth of algae. The analysis of effect of carbon dioxide on the growth of algae is complicated, because the effect of pH, HCO_3^-, CO_3^{--} and CO_2 distribution, hydration and dehydration rates, and metabolic functions of algae are coupled together. The presence of carbonic anhydrase enzyme in algae (Litchfield and Hood, 1964) which can catalyze the dehydration of HCO_3 further complicates the problem. The presence of enzyme in algal systems limited in carbon dioxide suggests that it is involved in carbon utilization, although its specific role in relation to inorganic carbon utilization in algae is not known.

In this chapter the effect of carbon dioxide feed rate, the mode of feeding carbon dioxide (carbon dioxide at 1 hr intervals for 1 hr), and addition of carbon dioxide using a pH controller on the growth of algae are examined.

a. EXPERIMENTAL METHODS

The efficiency of carbon utilization at different carbon dioxide feed rates was determined in 3 tanks for 1400 liter volume (urea 0.1 g/l, and Sequedin 0.2 g/l) by estimating the growth of algae and carbon dioxide put into the tank. All the tanks in a set were started with the same inoculum concentration. A polyethylene sheet of 400 x 75 cm was used in each tank over carbon dioxide supply pipe to avoid carbon dioxide losses from

culture to air. Absorbance of the culture was measured daily in the morning. The experiments with following conditions were performed to determine the gain in yield and carbon utilization efficiency due to continuous and intermittent feeding of carbon dioxide:

1. A control experiment where only air was used as sparger gas.

2. 250 ml/minute of pure carbon dioxide was continuously fed for the day light duration of the experiment (from 9 a.m. to 5 p.m.).

3. 500 ml/minute of pure carbon dioxide was continuously fed for the day light duration of the experiment.

4. 500 ml/minute of pure carbon dioxide was fed at one hour intervals for a one hour period during an 8 hours day, i.e. experiment (4) would use half the amount of gas in 8 hours as experiment (3), and the same amount of gas as experiment (2).

5. 1 liter/minute of pure carbon dioxide was continuously fed for the day light duration (8 hours) of the experiment.

6. 1 liter/minute of pure carbon dioxide was fed at one hour intervals for 1 hour period during an 8 hours day, i.e. experiment (6) would use half the amount of gas in 8 hours as experiment (5), and the same amount of gas as experiment (3).

7. Another set of experiments used a pH controller to operate a solenoid valve on carbon dioxide line. The pH was set at 8.3 and was maintained between 8.2 and 8.4. The flow

of carbon dioxide was 1 liter per minute at the on position of the controller and zero at the off position. The time and the frequency for which the controller was on were recorded.

8. The desorption of carbon dioxide at pH 8.3 was assumed zero, and the efficiency of carbon utilization was calculated on the basis of gain in yield over the control experiment due to carbon dioxide supply. The carbon in algae was assumed 50%, and the efficiency of carbon dioxide fed system was assumed 100%, i.e. the efficiency of carbon utilization reported includes the efficiency of carbon dioxide feed system also.

b. RESULTS

The absorbance versus time in days of one of the set is plotted in Figure (8.1). The efficiencies of carbon utilization, are plotted in Figure 8.2. The results of the experiment using controller to maintain the pH in algal culture are plotted in Figure 8.3. The times indicated on graph show the time for which the controller was on and off on the particular day.

Figure 8.4 and 8.5 show the change in pH and dissolved oxygen concentration at different time in tank with different treatments.

Following are the important points to note:-

1. The efficiency of carbon utilization decreases with increase in volumetric flow rate of carbon dioxide put into the culture. This may be seen from the three lower curves of Figure 8.2, where the continuous carbon dioxide fed runs are shown.

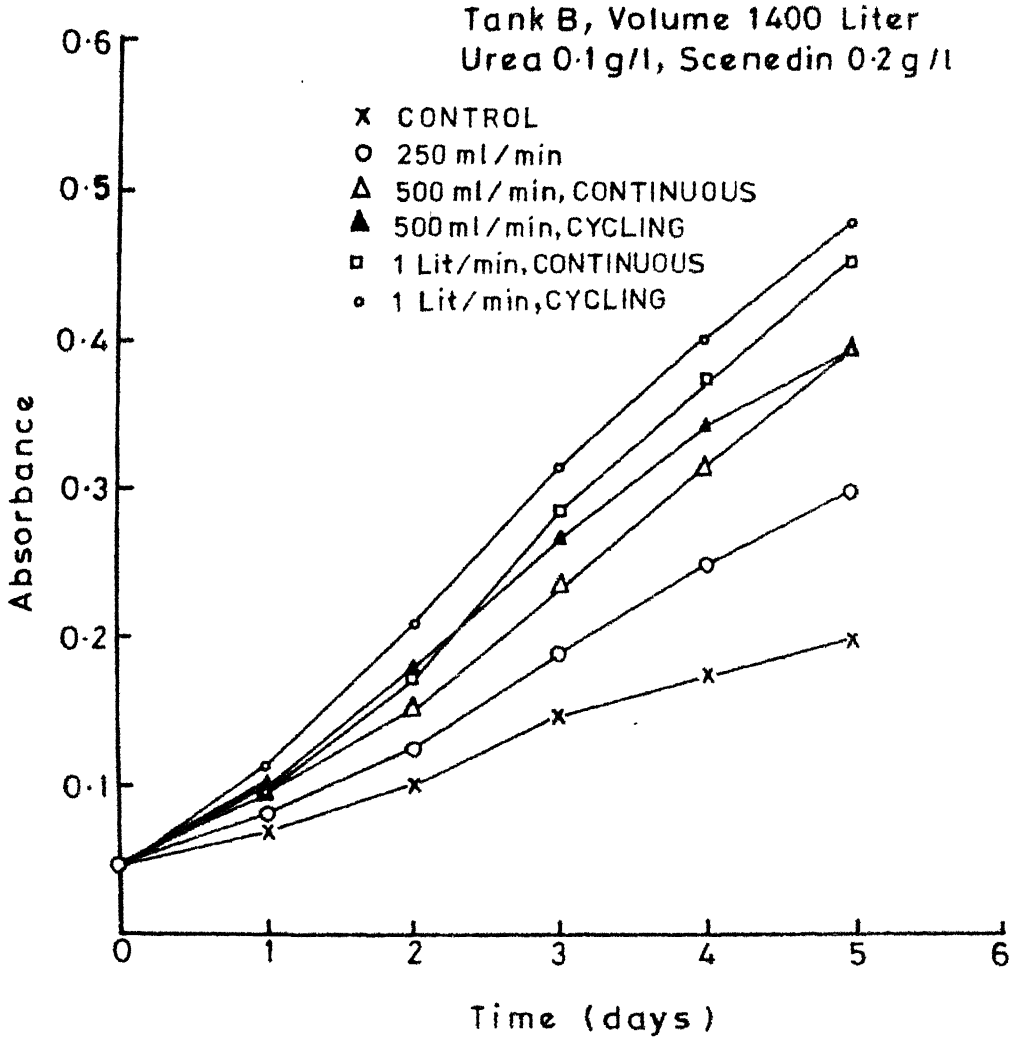


FIGURE 8·1 EFFECT OF CO_2 FEED RATE AND FEED METODS ON THE GROWTH OF ALGAE

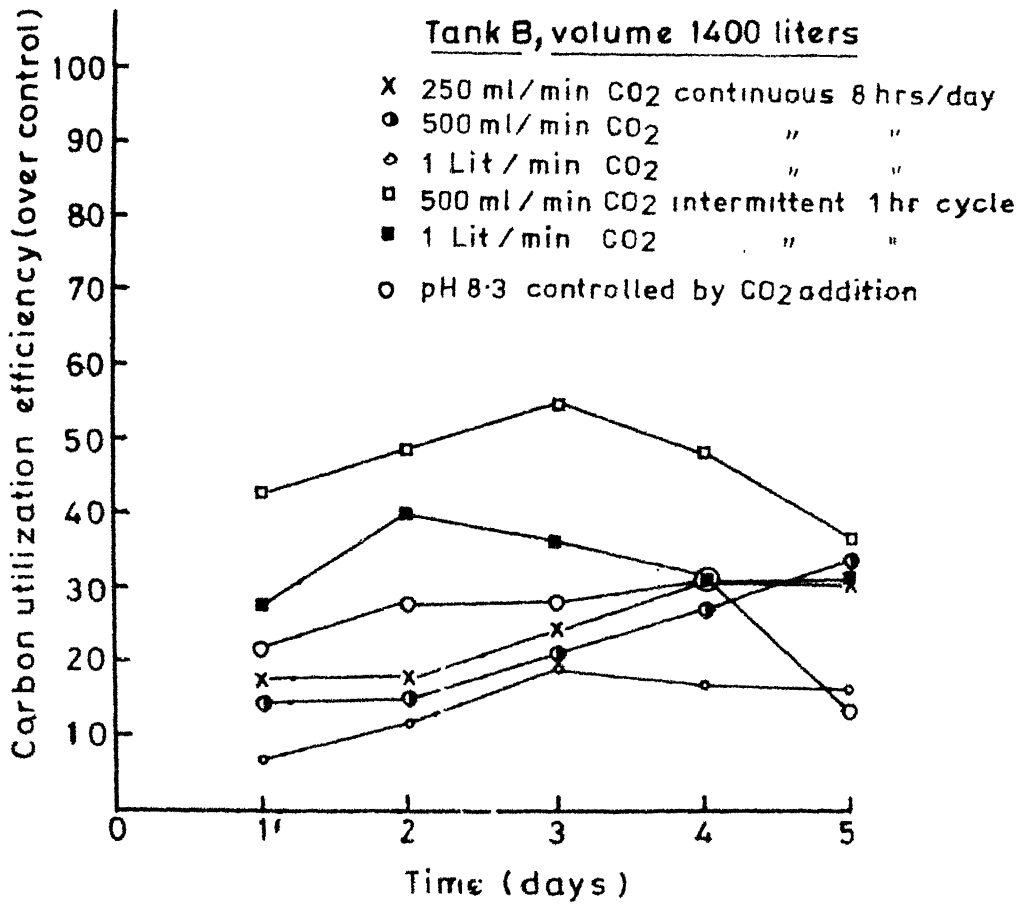


FIGURE 8·2 CARBON UTILIZATION EFFICIENCY OF ALGAE

2. The efficiency of carbon utilization when carbon dioxide flow is 250 ml/minute and 500 ml/minute, increases with time after second day, while efficiency of carbon utilization, when feed rate is 1 liter per minute, decreases after 3rd day.

3. The growth of algae with carbon dioxide fed at one hour intervals is always better than the growth in tanks getting an equal total amount of carbon dioxide continuously, whether judged on an efficiency basis or yield basis.

4. The growth in the tanks with intermittent feeding is better than the growth in the tank with continuous carbon dioxide supply (equal feed rate but double the total amount) for first few days. The gain in growth due to intermittency decreases as optical density increases.

5. The efficiency of carbon utilization in intermittent feeding is always higher than the efficiency in the continuous carbon dioxide feed experiments.

6. The growth in the tanks with 1 liter/minute intermittent carbon dioxide is greater than pH controlled addition (carbon dioxide), which, in turn, is greater than 1 liter/minute carbon dioxide fed continuously. The carbon dioxide fed in these three cases were 240 liter/day, 267 liter/day, and 480 liter/day, respectively.

7. The growth and efficiency of carbon utilization in tanks maintained at pH 8.3 by adding carbon dioxide (using a controller is lower than the efficiency in tank with 1 liter/minute intermittent feeding of carbon dioxide.

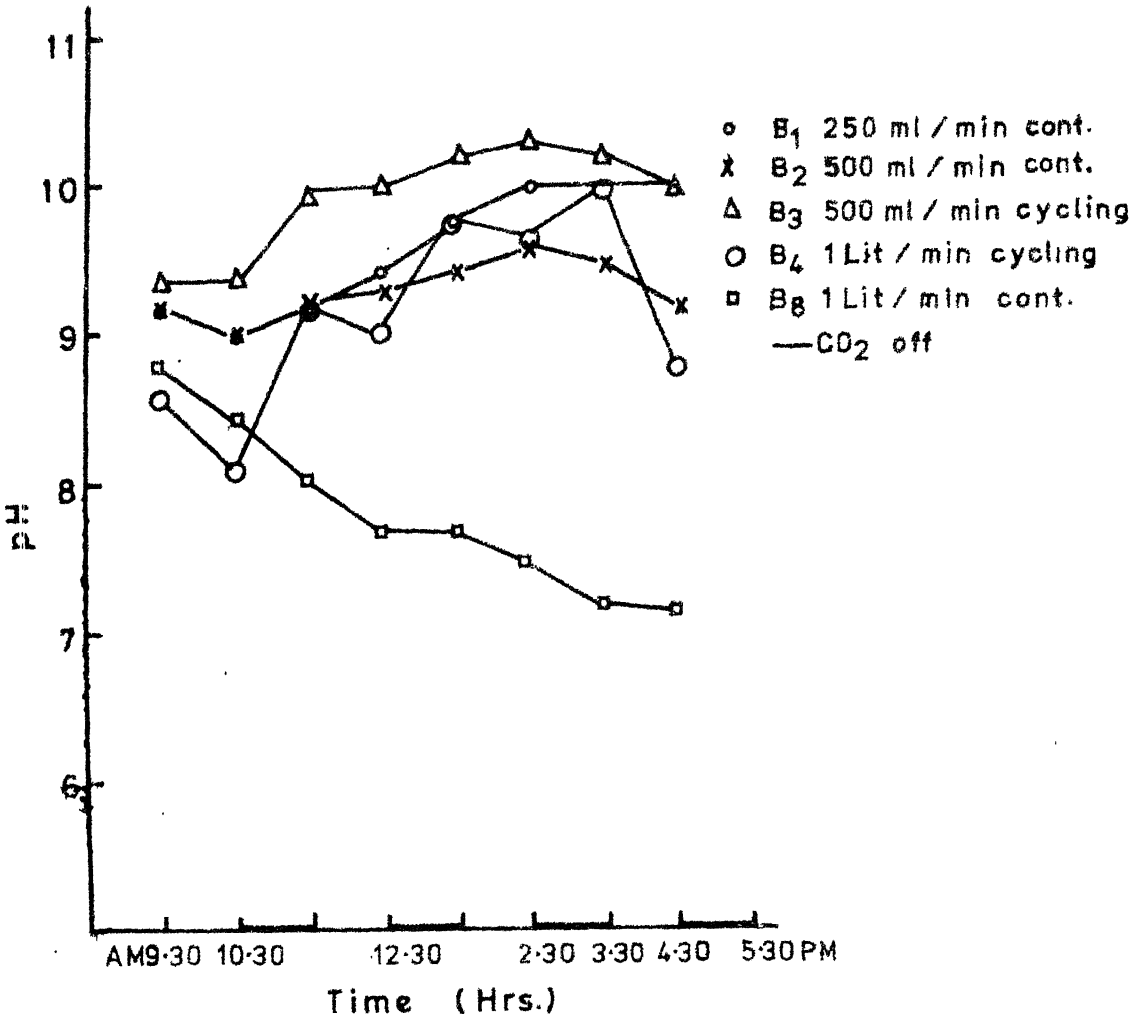


FIGURE 8·4 pH OF THE ALGAL CULTURES (DIFFERENT CO_2 FEED METHODS)

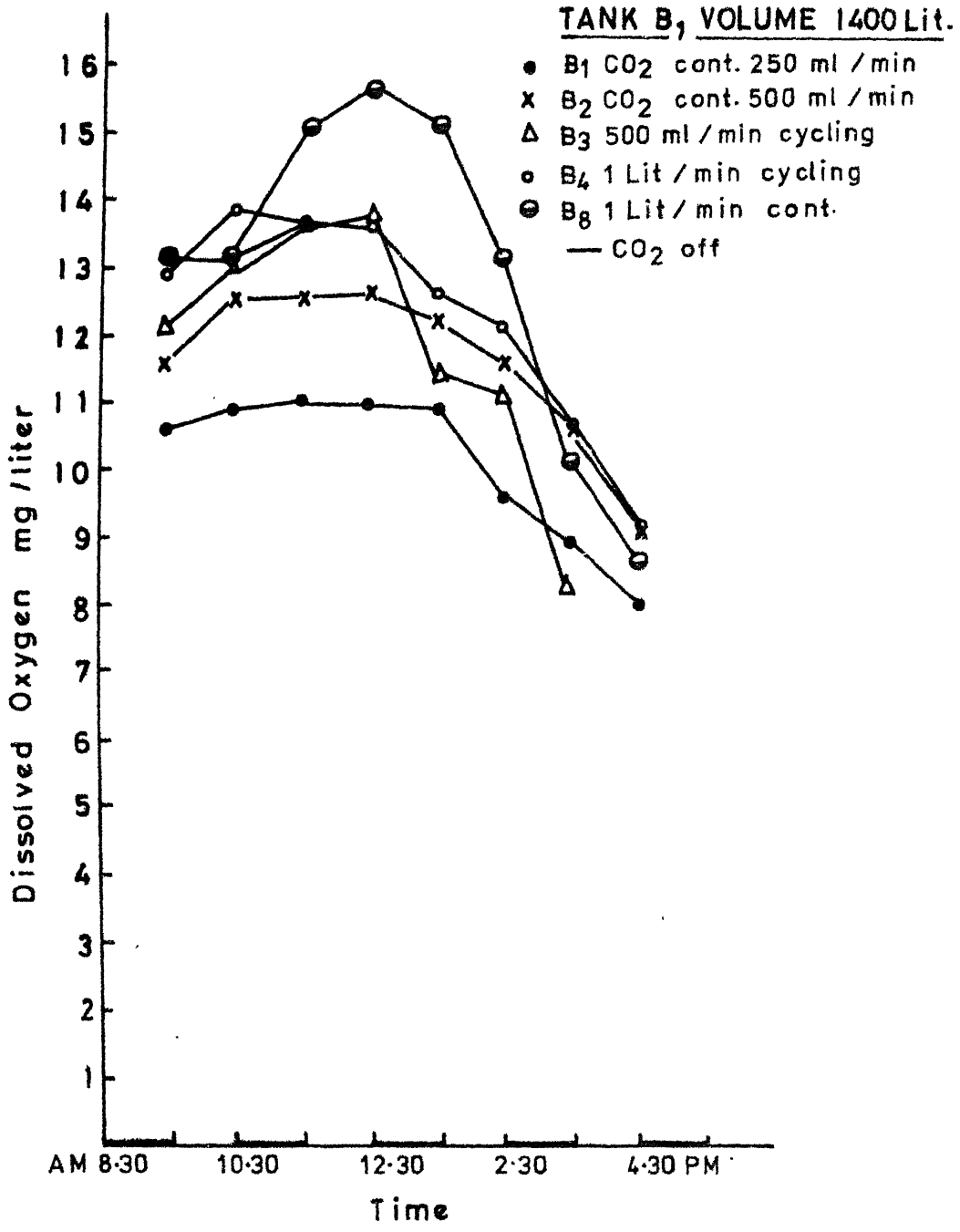


FIGURE 8·5 DISSOLVED OXYGEN OF ALGAL CULTURES (Different CO_2 feed methods)

8. Figure 8.6 shows the demand of carbon dioxide in percent at different interval of time of one day. From the figure it is clear that most of the demand (75%) is put in between 10 a.m. to 3 p.m. This leads to the conclusion that supply of carbon dioxide to algal cultures from 10 a.m. to 3 p.m. would be best utilized. This result can be extended further to expect better efficiency of carbon utilization by intermittent feeding of carbon dioxide from 10 a.m. to 3 p.m. This would further save on carbon dioxide budget.

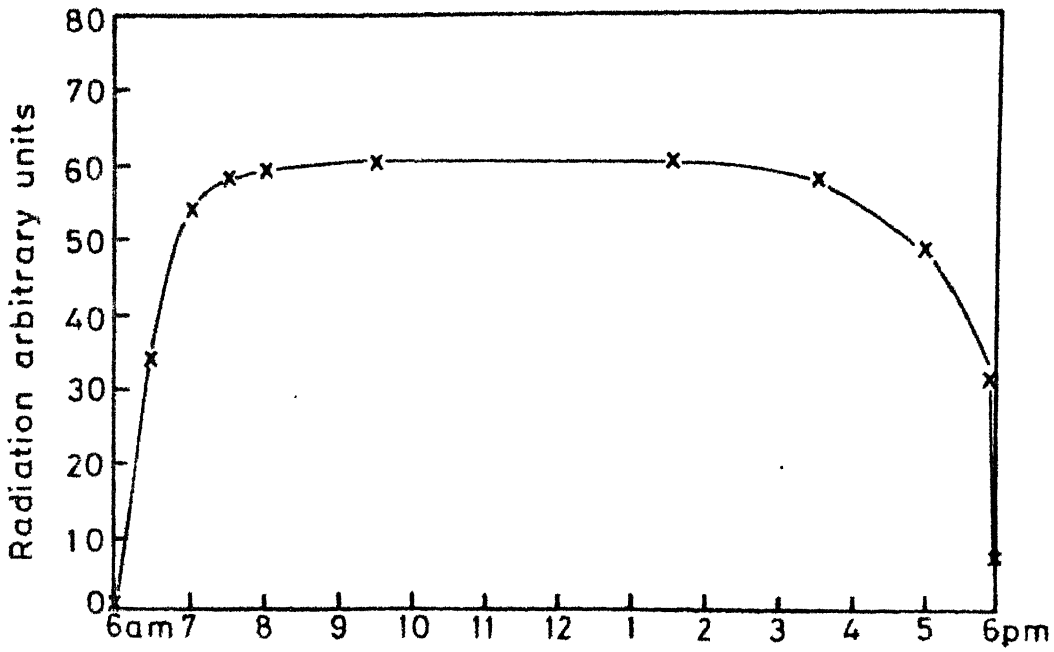
9. The following are the points observed from recorder chart (not shown here):

a. At low algae concentration, there is cycling of pH due to carbon dioxide cycling. At higher algal concentration the cycling behaviour of pH (due to carbon dioxide cycling) is lost due to higher carbon dioxide demand, and the difference in growt between intermittent and continuous tanks is reduced.

b. Dissolved oxygen concentration of algal culture changes almost without lag with light intensity, while change in dissolved oxygen due to pH, is slow and delayed.

c. DISCUSSION

The utilization of inorganic carbon in tanks with continuous supply of carbon dioxide seems to follows the law of diminishing returns. The efficiency is higher but growth is low at low carbon dioxide feed rates. Same rule is followed by algae in utilizing light. The efficiency of light utilization decreases with increase in light intensity.



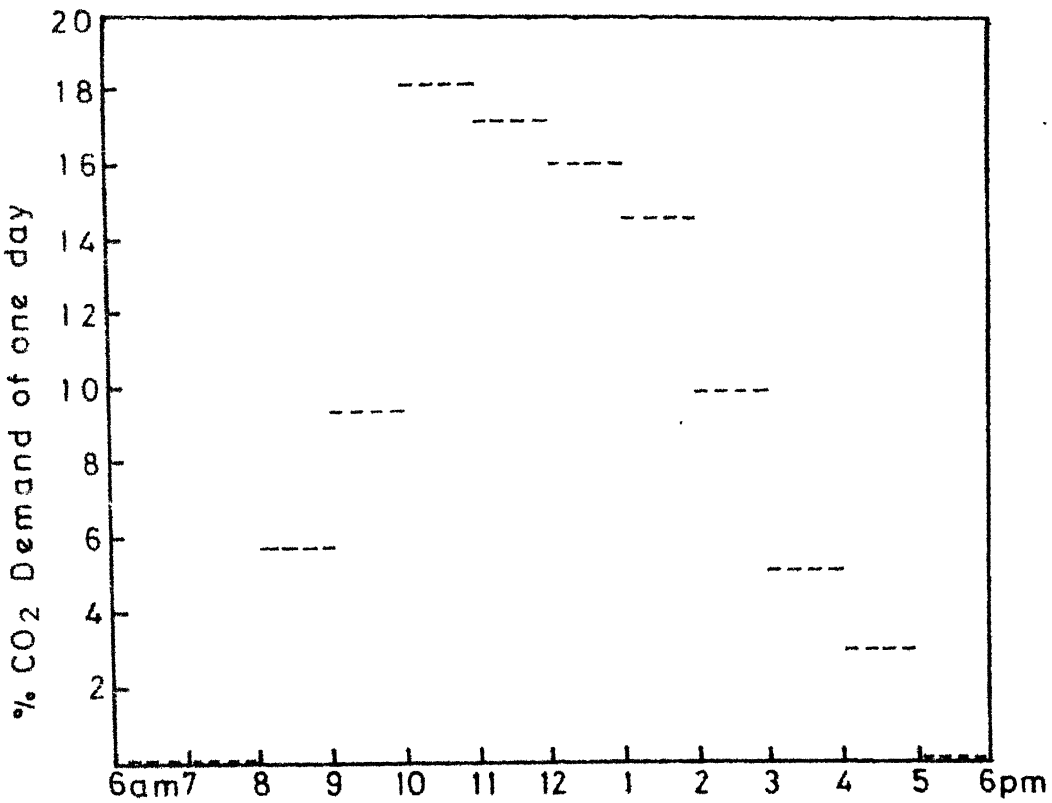


FIGURE 8·6 THE DEMAND OF CARBON DIOXIDE AT DIFFERENT TIME

The higher growth rate in intermittent-feed cultures may be the result of synchronization of the culture due to pH shocks. Probably increase in algal growth gains some momentum when carbon dioxide is fed, and this momentum is continued even when supply of carbon dioxide is stopped.

Another possible explanation for the increase in growth rate and carbon dioxide-carbon utilization efficiency is that enzyme carbonic anhydrogenase increases the efficiency and rate of carbon dioxide fixation. This enzyme can catalyse the dehydration of HCO_3^-. The concentration of enzyme is reported (Litchfield and Hood 1964) higher in the algae grown in carbon dioxide deficient media. Probably enzyme is active making HCO_3^- carbon available for growth of algae when there is no carbon dioxide supply and this activity continues at least for some time when carbon dioxide is supplied in intermittent pattern, giving higher yield and carbon utilization efficiency.

The interval of cycling (1 hr) and rates (500 ml/minute and 1 liter/minute) were arbitrarily chosen for our experiments. The optimum dosage and optimum frequency of carbon dioxide cycling determination is an area to be developed. Optimality criterion, and the growth cycle of algae may give some clue to determine the optimum conditions.

Goldman (1972) has suggested to include pH control through carbon dioxide addition in standard algal assay procedures so that data from different laboratories can be compared. Since mode of carbon dioxide addition also affects the growth, it is

here suggested to include in standard algal procedure, not only the addition of carbon dioxide but to fix the mode of carbon dioxide addition also.

d. CONCLUSION

The conclusions from this chapter are as follows:

1. The efficiency of carbon utilization decreases with increase in volumetric flow rate of carbon dioxide into the culture, when carbon dioxide is fed continuously.

2. The growth of algae and efficiency of carbon utilization in tanks getting carbon dioxide intermittingly at 1 hr intervals is higher than the growth and efficiency in tanks getting totally the same amount of carbon dioxide but continuously.

3. The efficiency of carbon utilization in tank maintained at pH 8.3 by adding carbon dioxide through a controller is less than the carbon utilization efficiency in tanks getting 1 liter per minute, and 500 ml/minute carbon dioxide at 1 hr interval for 8 hrs a day during day light.

4. 75% of the carbon dioxide demand of the day was put from 10 a.m. to 3 p.m. The control of pH and addition of carbon dioxide is very critical in this period. Intermittent feeding of carbon dioxide between 10 a.m. to 3 p.m. would further increase the carbon utilization efficiency.

5. The growth rates calculated at the end of 5th day are:

Treatment	Growth rate g/meter^2 day
CO_2 1 l/min - cycling	10.8
CO_2 1 l/min - continuous	10.18
CO_2 500 ml/min cycling	8.6
CO_2 500 ml/min continuous	8.6
CO_2 250 ml/min continuous	6.36
Control	3.8

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CHAPTER 9

EFFECT OF HARVESTING TIME ON PROTEIN CONTENT

The quality (wavelength), quantity, and mode of light affect the metabolic functions, growth and composition of algae. The effect of diurnally intermittent illumination, and flashing light on the growth and photosynthetic efficiency of algae are well known. The effect of blue and red light on the carbohydrate-protein ratio and respiration rate are reported by Kowallick (1970). The reduction in the productivity of algal cells to about 50% by giving light (20-25 lux) during dark phase are reported by Soeder, Schulze and Theile (1966) and Lorenzen and Foss (1968). The change in protein content in synchronized laboratory cultures in light-dark phase are reported by Tamiya (1964), Lorenzen (1970) and Jones et al., 1968.

Extending the results of Kowallick (1970) for blue and red light, and Lorenzen's (1970) for synchronous cultures to diurnal solar radiations and unsynchronized large scale outdoor cultures of Scenedesmus acutus, a change in protein content with time in light and dark phase was expected. This hypothesis was further extended to find out the optimum time for harvesting outdoor cultures to give higher protein content.

In this chapter, the effect of harvesting time on protein content of algae Scenedesmus acutus in outdoor cultures is presented.

a. EXPERIMENTAL METHODS

The experiments were performed in all sizes of tanks with the following treatments:

1) blank
2) blank + carbon dioxide
3) blank + molasses and
4) blank + carbon dioxide + molasses.

Tanks were prepared and samples were taken in the morning before sunrise and in the evening before sunset for protein estimation. 2-4 liter sample depending upon optical density of the culture was taken, filtered through fine cloth to remove contaminants (leaves, insects, etc.), centrifuged. and washed 4/5 times in distilled water. This slurry was used for dry weight estimation. and for nitrogen estimation. Nitroge was estimated by Kjeldahl method (Appendix 5.4). Samples were taken in duplicate and each sample was distilled twice. Protein is reported as nitrogen x 6.25.

To make sure of the above results, protein estimation by Biuret method (Herbert et al., 1971) was used. Biuret method was done on chlorophyll free white algal sludge. Chlorophyll was extracted by boiling algae in methanol for 5 minutes and was removed by centrifugation. Three to four extractions were

required to extract and remove all the chlorophyll. In an experiment algae was boiled in 3.0 N sodium hydroxide for 5, 10 and 15 minutes, and protein was determined to make sure that all the protein is extracted from algae by boiling it in 3 N NaOH for 5 minutes. Carbohydrate in algae was determined by anthrone method.

In an experiment, the thick algal slurry from centrifuge was taken in tubes, aerated and subjected to light and dark (12-12 hour) cycles. Protein was determined in algae before light, and before dark cycle. This experiment was carried out to find out the possibility of subjecting the thick algal suspension from centrifuge to light dark pattern in order to have the benefits of harvesting the algae in the morning, i.e., high protein in the algae.

The exact time of division in dark phase for algae reported in literature, is very conflicting. In an experiment it was tried to find out the time of division of Scenedesmus acutus in dark by counting the cell number using Haemocytometer. It was found that counting in samples depends upon the preparation of sample (may be due to fast settling rate of algae in Haemocytometer cubes) and gives 100-200% error in counting. Electronic counter cannot be used in this situation because 1) cells are not spherical, 2) they grow in singles, in fours and sometimes in singles and fours (we do not know the factors governing this behaviour of algae), 3) dust particles in open tank can be registered as cells in electronic counter.

b. RESULTS

Some of the important points to note are :-

I. Protein Estimation.

1. Boiling of algae in methanol for 5 minutes, thrice extracts all the chlorophyll.

2. Removal of chlorophyll by above method is easy when it is done on fresh algae. Storing the algae in refrigerator for 8-10 hours makes the extraction of chlorophyll very difficult.

3. Boiling 1 ml algal sludge in 2 ml 3 N sodium hydroxide for 5 minutes is enough to extract all the protein from the cells.

II. Protein Content

1. Protein content of algae in the morning before light cycle and in the evening before dark cycle are plotted in Figure 9,1 and 9.2.

2. Protein contents of algae are higher in the morning. There is 8 to 12% difference in protein content in the algae harvested before the light cycle and after the light cycle.

3. The protein contents estimated by biuret method were lower (30-50% protein in algae) than estimated by Kjeldahl method (50-60%). However, there was 8-12% difference in protein content of algae harvested in the morning and in the evening by biuret method.

4. This difference in protein content was observed in all

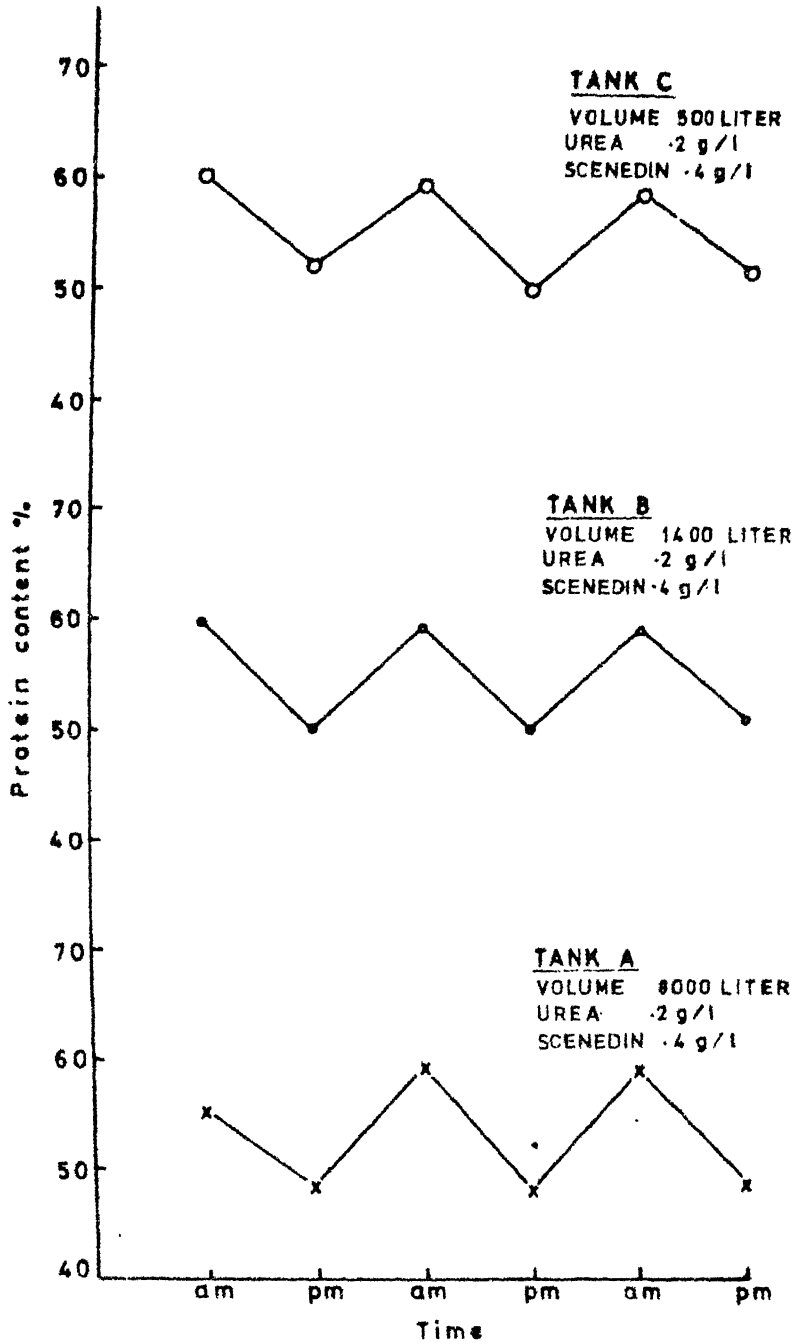

FIGURE 9·1 EFFECT OF HARVESTING TIME ON PROTEIN CONTENT (Different tanks)

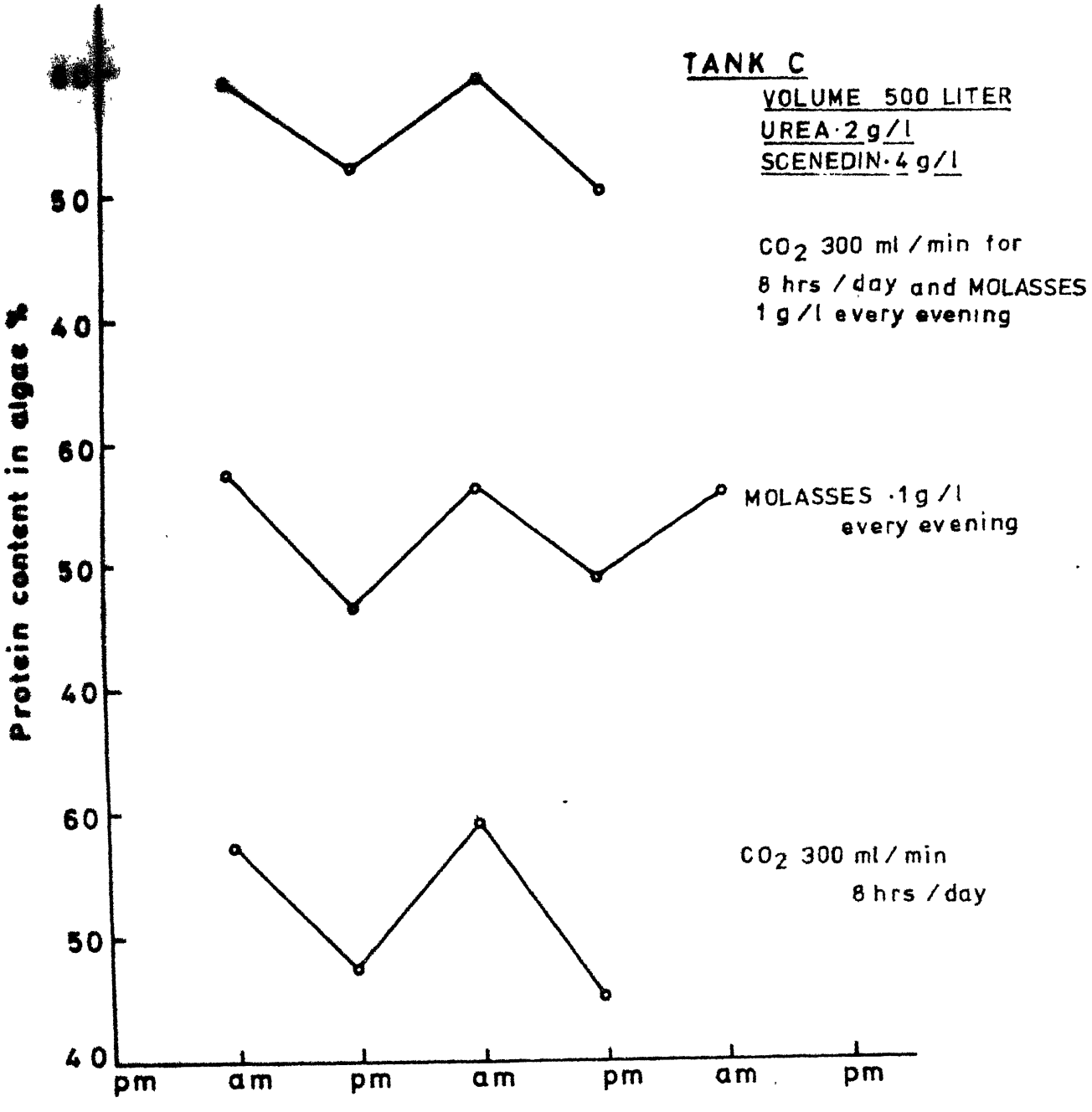

FIGURE 9·2 EFFECT OF HARVESTING TIME ON PROTEIN CONTENT (Different treatment)

the treatments and in all the tanks.

5. There was difference in carbohydrates in algae in the morning and in the evening (lower in the morning), but this difference was small and never satisfied the material balance.

6. There was no difference in protein content in light-dark phase in centrifuged washed (and thus difficient in nitrogen and other nutrients) thick algal suspension subjected to 12 hr light, 12 hr dark cycle.

7. Difference in protein in % of protein present, is plotted in Figure 9.3 against optical density. From the Figure it seems that difference in protein is independent of optical density of algal culture.

c. DISCUSSION

It is very difficult to explain the exact mechanism of the effect of light-dark on the protein content of algae, observed in the above experiments. A question can be asked. Does the dark enhances the protein synthesis and carbohydrate break down or the light suppresses the above reactions ? The explanation given by Mowallick (1970) for blue and red light effect can be extended to say that probably dark enhances the protein synthesis, and the energy and carbon for protein synthesis is provided by breakdown of carbohydrates. Another simple explanation is that in day time during photo-synthesis carbon and nitrogen, both are assimilated to give carbohydrate and protein. In dark, in the absence of

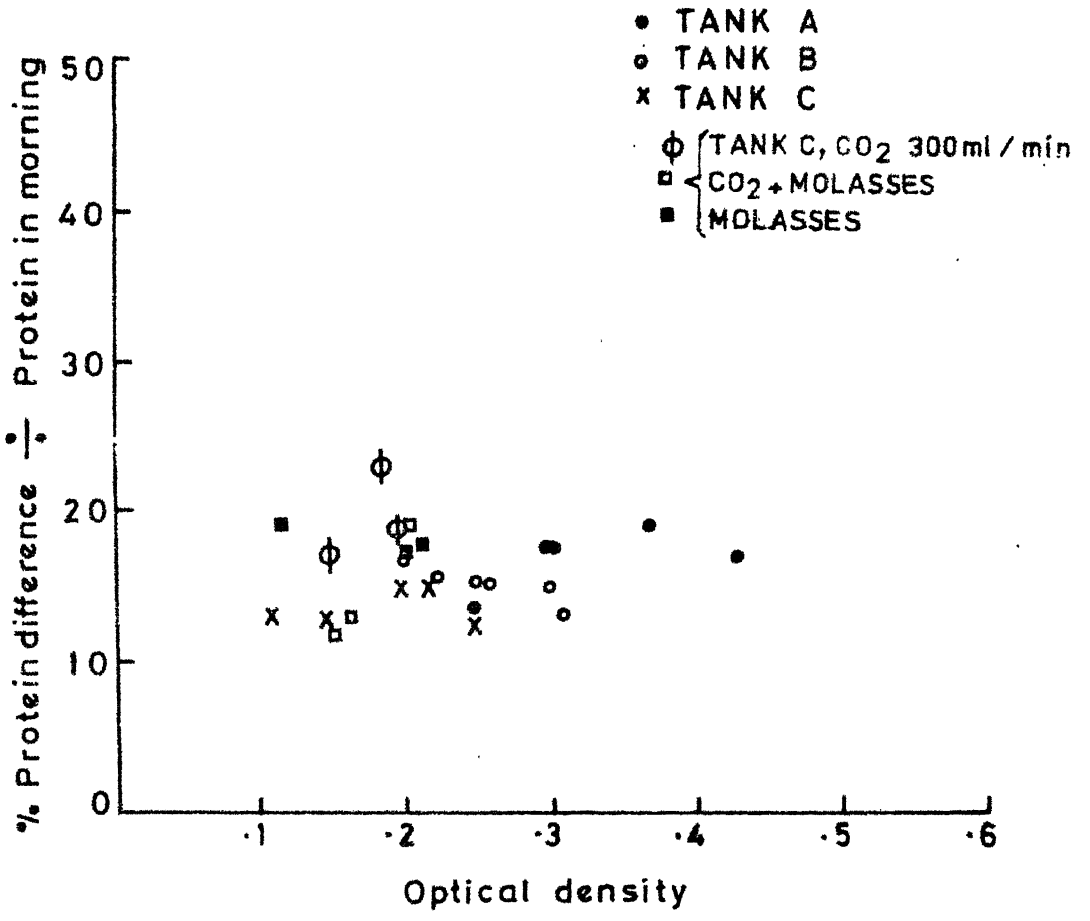

FIGURE 9·3 PROTEIN DIFFERENCE (AM to PM) % VERSUS OPTICAL DENSITY

photosynthesis there is no intake of carbon (there are losses of carbon due to the respiration), but there is transfer of nitrogen from medium to algal cells; increasing the nitrogen content, and hence protein synthesis which is independent of light (see Lorenzen, 1970). In other words, the culture may be nitrogen limiting in light but at night, cultures are definitely carbon limiting and this results in more protein in algae cells after dark phase. This hypothesis is supported by the results of the experiment in which there was no difference in protein content in nitrogen deficient cultures in light and dark phase cells.

Results of this experiment suggests that algae for protein should be harvested in the morning time before sunrise. This time of harvesting would give 10-15% more protein than that harvested in day time. The other advantage of harvesting in the morning is that in the morning the temperature of the culture is low, and at this lower temperature all the enzymatic activities are low. The losses (respiration losses) and enzymatic changes will be low at low temperature in the morning.

The above results can be extended to suggest that algae for the production of energy (to produce methane by anaerobic digestion) should be harvested after light period. This would give higher carbohydrate containing-algae for energy production.

CONCLUSION

1. Protein content of the algae, harvested in the morning before light, is 10-15% more than the protein in the algae

harvested in the evening.

2. The above difference in the protein content is true in tanks, without external carbon dioxide supply, with carbon dioxide, and with organic carbon adding in the evening.

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SUMMARY AND CONCLUSIONS FROM EXPERIMENTS OF PART II

In this part, which deals with fine parameters, following studies were carried out:

1. The effect of addition of organic carbon on the growth of algae in outdoor and indoor cultures.
2. Estimation of dispersion coefficient in tanks.
3. Carbon dioxide transfer from algal culture and its correlation with tank parameters.
4. Effect of carbon dioxide and carbon dioxide feed methods on the growth of algae.
5. Effect of harvesting time on protein content.

The major conclusions from Part II are as follows:

1. There was an increase in the growth by addition of molasses in the evening into the algal tanks. There was almost no increase in biomass at night, however, the absorbed sugar was utilised for the day time growth.
2. The absorption of sugar into the algal biomass in night was found much more rapid than its utilization for cell synthesis in day.
3. The effect of addition of molasses and carbon dioxide on growth was cumulative. This was true for low concentration of molasses (about 0.1 g/l).

4. 95% of the molasses carbon goes into the algae. This leads to the conclusion that addition of molasses does not increase the respiration rate of Scenedesmus acutus.

5. The absorption of sugar in night follows a first order kinetics with respect to sugar, and absorption rate constant depends upon algae concentration.

6. The addition of molasses intermittingly leads to significant increase in growth in indoor cultures.

7. Average velocity of liquid in tank increases with the increase in liquid height.

8. Dispersion coefficient increases with increase in liquid height.

9. Reynolds number based on hydraulic diameter was found always greater than 590×10^2. This Reynolds number for open channels is in turbulent flow regime.

10. Peclet number based on hydraulic diameter and longitudinal mixing coefficient was between 1 to 3 in most of the cases.

11. Hydrodynamically C tanks are very much different from A and B tanks.

12. The desorption of carbon dioxide from the tanks can be obtained by assuming it as a 1st order batch process. Mass transfer coefficient in B and A tanks was found reasonably constant at 0.0029 cm/sec. The Sherwood number was related to the Reynolds number as:

$$Sh = 0.037\ Re^{0.83}$$

13. The efficiency of carbon utilization decreased, and growth increased with increase in carbon dioxide volumetric flow rate in to the culture when it was fed continuously.

14. The growth of algae and efficiency of carbon utilization in tanks getting carbon dioxide intermittingly at 1 hr intervals, for 1 hour, was higher than the growth and efficiency in tanks getting the same amount of total carbon dioxide.

15. The efficiency of carbon utilization in tanks controlled at pH 8.3 by adding carbon dioxide was lower than the efficiency in tanks getting carbon dioxide at the rate of 1 liter/min and 500 ml/min, intermittingly at 1 hr intervals.

16. Protein content of the algae harvested in the morning before light period was 10-15% more than the protein in the algae harvested after light period. This difference in protein was found in tanks with external carbon dioxide supply, in tanks with addition of molasses in the evening, and in all the sizes of tanks.

APPENDIX 1

1.1. CHEMICAL COMPOSITION OF ALGAE SCENEDESMUS ACUTUS

(Becker et al., 1976)*

Component	Range %
Protein	50-55
Lipids	12-19
Carbohydrates	10-15
Fibre	10-12
Ash	6-8
Nucleic Acid	4-6
Moisture	5-7

1.2. PROTEIN EFFICIENCY RATIO (PER), BIOLOGICAL VALUE (BV), DIGESTIBILITY COEFFICIENT (DC), AND NET PROTEIN UTILISATION (NPU) OF ALGAE SCENEDESMUS ACUTUS

(Becker et al., 1976)*

No.	Processing and diet	Level of Protein	PER	% Nitrogen retained	BV	DC	NPU
1	Casein	10	2.78	76.64	87.76	95.08	83.54
2	Algae DD	10	2.21	58.41	80.82	81.42	65.80
3	Algae DD + Methionine	10	2.45	-	-	-	-
4	Algae DD	20	1.87	47.01	67.13	77.40	51.95
5	Algae SD	10	1.27	40.68	72.07	72.50	52.25
6	Algae CSD	10	1.34	51.85	71.91	77.14	55.47

DD Drum Dried; SD Sun Dried; CSD Cooked Sun Dried

*Becker, W.E., Venkataraman, L.V. 1976. 'Food for Algae' IGAP, CFTRI, Mysore.

APPENDIX 2

PRODUCTIVITY OF ALGAE AND OTHER PROTEIN SOURCES (Vincent 1971)[x]

Protein Source	Yield dry wt. of protein kg/ha/yr	Area yielding 29.2 g protein/y^2 (m^2)
Scenedesmus Acutus*	37,500	7.8
Clover leaf protein	1,680	174
Wheat	300	970
Milk from cattle on grass land	100	2910
Meat from cattle on grass land	60	4870

*Calculated on the basis of 25 gm algae/m^2 day; 50% Protein

[x] Vincent, W.A., 1971, 'Microbes and Biological Productivity' 21st Symp. of the Soc. of Gen. Microbiology, ed. Hugens D.E. and Rose, A.H., :47.

APPENDIX 3

EFFECT OF INOCULATION OF AULOSIRA FERTILISSIMA ON AVERAGE GROWTH AND YIELD OF A VARIETY OF PADDY-TA (SINGH R.N. 1961)[+]

Experiment	Height of plants cm	Yield paddy per pot gm	Yield of paddy per acre lb	% increase over control
Control	73	5.0	1,060	-
Pots (inoculated)	141	23.4	-	368.0
Fields (inoculated)	135	-	2,277	114.8

[+]Singh R.N., 1961. 'Role of Blue Algae in Nitrogen Economy of Indian Agriculture', ICAR, New Delhi: 30.

APPENDIX 4

COMPOSITION OF SCENEDIN*

NH_4-N	9.45%	Mn	.08 %
K_2O	15.6 %	Ni	40 ppm
P_2O_5	24.9%	Co	8 ppm
Mg	2.74%	Zn	200 ppm
SO_4	13.5%	V	2.4 ppm
Cl	12.7%	Cu	24 ppm
Na	1.39%	Pb	3.5 ppm
Fe	2.0%		

Based on the information of the manufacturer Chemische Werke Gewerkschaft Victor, Federal Republic of Germany.

APPENDIX 5

CHEMICAL ANALYSIS

5.1. ESTIMATION OF SUGARS IN MEDIUM

PHENOL SULPHURIC ACID METHOD - (Dubois Michel, K.A.Gilles, J.K. Hamilton, R.A. Peter, and Freed Smith, 1956. Anal. Chem. 28:3500)

METHOD - 1 ml centrifuged clear sample was taken in tube and to it was added 1 ml 5% phenol. 5 ml concentrated sulphuric acid was added rapidly using wide mouth pipette. The contents were kept at room temperature for 15 minutes, were shaken, and absorbance was taken at 490 nm against a blank.

5.2 ESTIMATION OF SUGAR IN ALGAE

ANTHRONE METHOD

REAGENTS - 660 ml H_2SO_4 was added to 340 ml water. 500 mg anthrone and 10 g thiourea were added to this sulphuric acid. The mixture was maintained at 80-90°C till all the solutes were dissolved. This reagent is stable for two weeks at 4°C.

METHOD - Centrifuged and washed algae (1-1.5 g) were hydrolyzed in 30 ml 1.5 N H_2SO_4 for 3 hours in boiling water. After cooling, the extract was neutralized with NaOH, and volume was made up to 100 ml with distilled water. The contents were centrifuged, and 1 ml of this clear solution taken in a test tube was kept in freezing mixture. 10 ml anthrone solution was added and content were mixed keeping the tubes in freezing mixture. Samples were kept in boiling water for exactly

15 minutes for colour development. The reaction was stopped by keeping the tubes in freezing mixture and then tubes were kept in dark for 10 minutes at room temperature. The carbohydrate in samples were estimated by measuring the colour developed at 620 nm.

5.3 BIURET METHOD FOR PROTEIN ESTIMATION

(Hrebert et al., 1971. 'Methods in Microbiology' ed. Norris Jr. and Ribbons, D.W., Academic Press:244)

METHOD - The sample (containing 2-10 mg dry algae) was centrifuged, and algae cells were boiled in 10 ml methanol for 5 minutes. Chlorophyll extracted was removed by centrifuging the methanol-algae suspension, and extraction was repeated to get chlorophyll-free algal sludge.

1.0 ml, 3 N NaOH was added to the above sludge after making up the volume to 2 ml with distilled water. Contents were boiled in boiling water for 5 minutes, and then were cooled to room temperature. 1.0 ml 2.5% $CuSO_4$ solution was added, contents were mixed well, and were allowed to stand for 5 minutes. Optical density of the centrifuged supernatent was taken 555 nm. A reagent blank containing 2 ml distilled water instead of cell suspension was treated in the same way.

5.4. KJELDAHL METHOD FOR NITROGEN ESTIMATION

A known volume of centrifuged and washed slurry was transferred to Kjeldahl's digestion flask, and 25 ml H_2SO_4 and a pinch of selene digestion mixture was added. The sample was digested on heating mantel for about 6 to 8 hours or till it was clear. The contents were diluted and volume was made up to 100 ml. 2 to 5 ml of this solution was used for ammonia distillation. The ammonia gas was liberated by adding 30 ml 40% NaOH, and heating the contents. The ammonia liberated was absorbed in 10 ml boric acid and was titrated against N/70 HCl. A known amount of ammonium salt was distilled and titrated to calculate the conversion factor.

5.5 CARBON DIOXIDE CONCENTRATION ESTIMATION

(Deutsche Einheitsuertahren Zur. Wasser Und Schlamn Untersuchung, D-8 Verlag Chemic Weinheim/Bergster, 1972)

The concentration of carbon dioxide in tap water was measured by determining the amount of NaOH solution consumed to bring the pH upto 8.3. In all the estimations, CO_2 free distilled water was used. The CO_2 concentration was checked by using phenolphthalein indicator following the method given in 'Standard Methods for the Examination of Water and Waste Water', American Water Works Association, Water Pollu., Cont. Fed. Pub., 13th ed., page 92, 1971.

$$\text{mg/l } CO_2 = \frac{A \times N \times 44{,}000}{\text{ml of Sample}}$$

Where, A = ml of NaOH consumed.

N = Normality of NaOH.

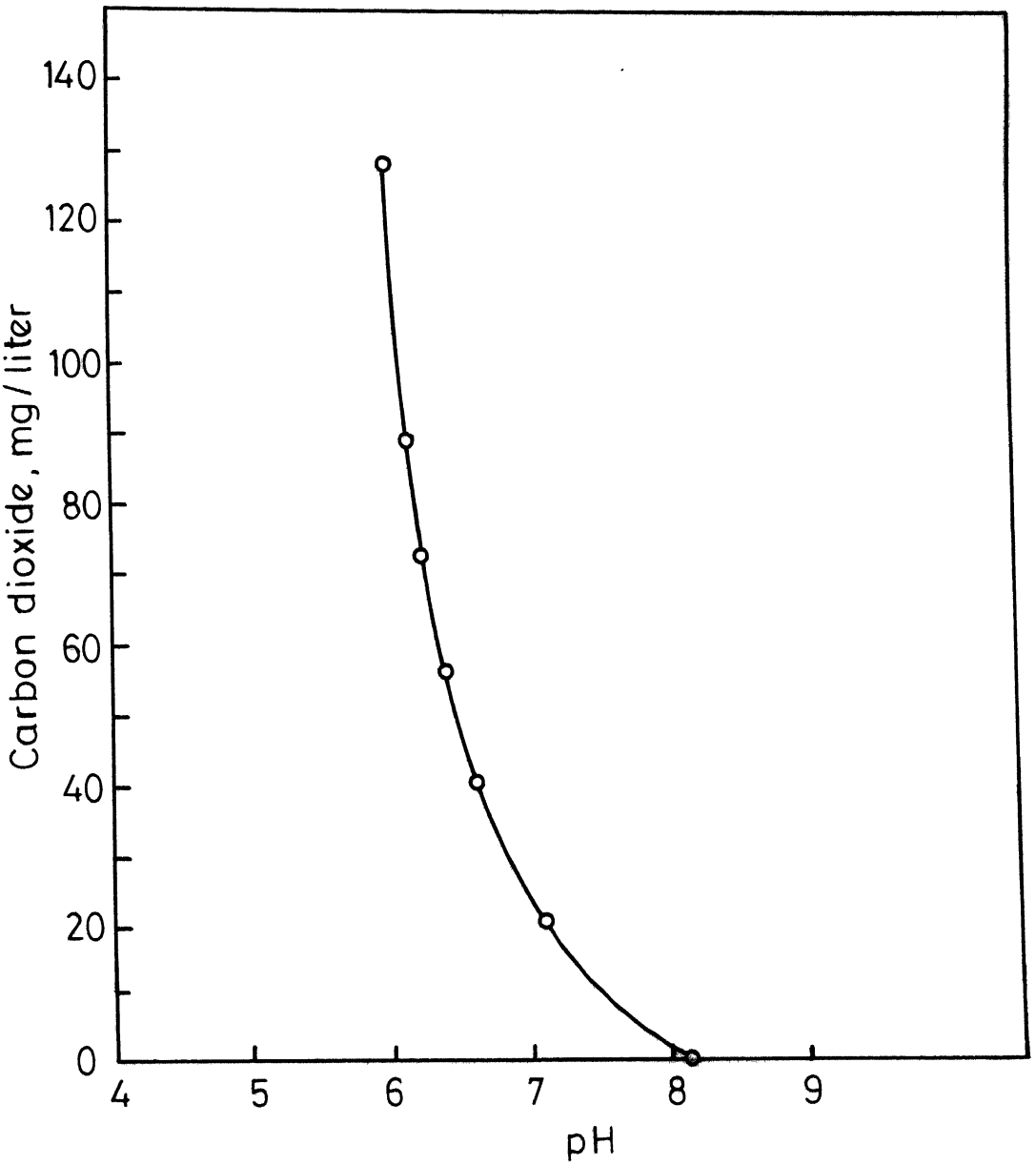

Fig. (Appendix 5-5) - Standard curve pH vs. CO_2.

APPENDIX 6

HEAT TRANSFER COEFFICIENT OF DRUM DRIER

Basis - 1 hr operation at drum drier.

Dry solids in feed	=	3.47%
Moisture in dried final product.	=	8.8 %
Product dried from drum drier/hr.	=	371.8 gm/hr
Feed for this	=	9750 gms
Water evaporated	=	9379.4 gms.

Heat required

(1)	To raise the temp. of slurry from room temp. 27°C to 100°C	=	9750 x 73
		=	702,000 cal.
(2)	Latent heat	=	9379.4 x 539.5
		=	50,60,000 cal.
	Total heat	=	57,60,000
	Area of drum	=	$.55 \times 10^4$ cm^2
	Temperature difference	=	110-27 = 83°C
	Heat transfer coefficient	=	12.66 $cal/cm^2.hr,°C$

APPENDIX 7

MIXOTROPHIC GROWTH DATA

Tank	Time	hrs	OD	Sugar So γg/ml	Sugar S γg/ml	S/So	x X t OD x hr
Tank C_2	7 PM	0	.115	126.5		1	0
Vol. 600 l	9 PM	2	.13		122.5	.97	.26
October	11 PM	4	.125		97.5	.78	.5
(Glucose)	1 AM	6	.13		89.0	.70	.78
	3 AM	8	.13		78.0	.61	1.04
	5 AM	10	.13		55.5	.44	1.3
	6 AM	11	.14		52.0	.41	1.54
Tank C_3	7 PM	0	.111	126.2	126.2	1	0
Vol. 600 l	9 PM	2	.12		126.2	1	.24
October	11 PM	4	.12		100.0	.79	.48
(Glucose)	1 AM	6	.125		92.5	.73	.75
	5 AM	10	.13		59.0	.47	1.3
Tank C_2	7 PM	0	.25	50.8	50.8	1	0
Vol. 600 l	11 PM	4	.25		25.0	.5	1.0
October	7 AM	12	.25		7.4	.14	3.0
(Molasses)							
Tank C_3	7 PM	0	.25	50.8	50.8	1	0
Vol. 600 l	11 PM	4	.25		25.0	.50	1.0
October	7 AM	12	.25		8.4	.165	3.0
(Molasses)							
Tank A_2	7 PM	0	.24	56.2	56.2	1	0
Vol. 8000 l	11 PM	4	.23		31.8	.56	.92
October	7 AM	12	.24		11.0	.19	2.9
(Molasses)							

Tank	Time	hrs	OD	Sugar So γg/ml	Sugar S γg/ml	S/So	x x t OD x hr
Tank B_6	7 PM	0	.277	43.0	43.0	1.0	0
Vol. 1400 l	11 PM	4	.26		27.4	0.63	1.04
October	7 AM	12	.26		6.45	0.15	3.12
(Molasses)							
Tank B_7	7 PM	0	.121	45.2	45.2	1.0	0
Vol. 1400 l	11 PM	4	.115		38.4	0.84	0.46
Molasses + CO_2	7 AM	12	.12		11.0	0.24	1.4
Tank B_2	1st day	15	.1	94.5	30.0	0.32	1.5
Vol. 1000 l	2nd day	15	.175	63.0	11.0	0.17	2.6
	3rd day	15	.24	94.5	11.0	0.12	3.6
Molasses	4th day	15	.33	94.5	3.0	0.03	4.95
5PM - 8 AM							
Tank B	1st day	15	.105	94.5	27.0	0.285	1.57
Vol. 1000 l	2nd day	15	.185	63.0	9.0	0.14	2.77
Molasses +	3rd day	15	.27	94.5	11.0	0.11	4.05
CO_2	4th day	15	.35	94.5	2.0	0.02	5.25
5PM - 8 AM	5th day	15	.39	94.5	3.0	0.03	5.85
Tank B_2	1st day	15	.32	70.0	6.5	0.09	4.8
Vol. 1300 l	2nd day	15	.43	105.0	7.0	0.07	6.4
Molasses							
5 PM - 8 AM							
Tank B	1st day	15	.21	105.0	6.5	0.06	3.15
Vol. 1300 l	2nd day	15	.26	105.0	8.0	0.076	3.9
Molasses							
5 PM - 8 AM							
Tank B	1st day	15	.04	70.0	12.0	0.17	0.6
Vol. 1300 l	2nd day	15	0.1	70.0	22.0	0.31	1.5
Molasses	3rd day	15	.14	70.0	15.0	0.21	2.1
5 PM - 8 AM							